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EVIDENCE FOR POPULATION BOTTLENECKS AND SUBTLE GENETIC STRUCTURE IN THE YELLOW RAIL

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Abstract. The Yellow Rail (*Coturnicops noveboracensis*) is among the most enigmatic and least studied North American birds. Nesting exclusively in marshes and wetlands, it breeds largely east of the Rocky Mountains in the northern United States and Canada, but there is an isolated population in southern Oregon once believed extirpated. The degree of connectivity of the Oregon population with the main population is unknown. We used mitochondrial DNA sequences (mtDNA) and six microsatellite loci to characterize the Yellow Rail's genetic structure and diversity patterns in six areas. Our mtDNA-based analyses of genetic structure identified significant population differentiation, but pairwise comparison of regions identified no clear geographic trends. In contrast, microsatellites suggested subtle genetic structure differentiating the Oregon population from those in the five regions sampled in the Yellow Rail's main breeding range. The genetic diversity of the Oregon population was also the lowest of the six regions sampled, and Oregon was one of three regions that demonstrated evidence of recent population bottlenecks. Factors that produced population reductions may include loss of wetlands to development and agricultural conversion, drought, and wildfire. At this time, we are unable to determine if the high percentage (50%) of populations having experienced bottlenecks is representative of the Yellow Rail's entire range. Further genetic data from additional breeding populations will be required for this issue to be addressed.

Key words: Yellow Rail, *Coturnicops noveboracensis*, genetic structure, bottlenecks, disjunct population, genetic diversity.

Evidencia de Cuellos de Botella Poblacionales y Estructura Genética Sutil en *Coturnicops noveboracensis*

Resumen. *Coturnicops noveboracensis* es una de las aves más enigmáticas y menos estudiadas de América del Norte, que nidifica exclusivamente en los pantanos y los humedales. Nidifica principalmente al este de las Montañas Rocallosas en el norte de Estados Unidos y Canadá, pero existe una población aislada en el sur de Oregon que anteriormente se suponía extirpada. El grado de conectividad de la población de Oregon con la población principal es desconocido. Empleamos secuencias de ADN mitocondrial (ADNmt) y seis loci de los microsatélites para caracterizar la estructura genética y los patrones de diversidad de *C. noveboracensis* en seis áreas. Nuestros análisis de la estructura genética basados en ADNmt identificaron una diferenciación poblacional significativa, pero la comparación pareada de las regiones no identificó tendencias geográficas claras. En contraste, los microsatélites sugirieron una estructura genética sutil que diferenció la población de Oregon de aquellas de las cinco regiones muestreadas en el área reproductiva principal de *C. noveboracensis*. La diversidad genética de la población de Oregon fue también la más baja de las seis regiones muestreadas, y Oregon fue una de las tres regiones que brindó evidencias de cuellos de botella poblacionales recientes. Los factores que produjeron reducciones en las poblaciones pueden incluir pérdidas de humedales por expansión urbana y conversión a agricultura, sequía e incendios de ambientes silvestres. En este momento, no somos capaces de determinar si el alto porcentaje (50%) de poblaciones que han sufrido cuellos de botella es representativo del rango completo de *C. noveboracensis*. Se requerirán datos genéticos adicionales de poblaciones reproductivas para analizar este asunto.

INTRODUCTION

Breeding in shallow wetlands and marshes, the Yellow Rail (*Coturnicops noveboracensis*) is the sole representative of the genus *Coturnicops* in North America (Taylor 1998). The majority of the species' summer range lies east of the Rocky

Mountains. In Canada, breeding populations have been documented from Saskatchewan to the Atlantic coast (Bookhout 1995). In the United States, the species breeds primarily in Maine, Michigan, Wisconsin, Minnesota, North Dakota, and northeastern Montana (Bookhout 1995). It migrates from this

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breeding range to winter in the southeastern U.S. along the coasts of the Gulf of Mexico and Atlantic Ocean.

Of particular interest is a geographically isolated breeding population in the western U.S. There are records of the Yellow Rail breeding in Mono County, California, from the 1920s to 1950 (McCaskie et al. 1980), and breeding was confirmed in Klamath County in south-central Oregon in 1926 (Griffiee 1944, Contreras 1993). Over the 30 years from 1950 to 1980, however, the species was not detected in the region, leading to speculation that the Yellow Rail had been extirpated from the West (American Ornithologists' Union 1983). Reports of the species' distinctive call emerged in 1982 and were followed by new documentation of the Yellow Rail breeding in Klamath County over the next several years (Stern et al. 1993, Popper and Stern 2000). Reports also indicate that Yellow Rails inhabit northern California year round (Sterling 2008), with birds occasionally observed during the breeding season in coastal marshes and wetlands where they winter. Dawson (1923) noted "scores" of Yellow Rails wintering in San Francisco Bay marshes, but more recently Sterling (2008) reported that only "small numbers winter in a few coastal marshes." Birds that breed in Oregon may also winter along the California coast.

In this study, we report results of the first analysis of the Yellow Rail's genetic diversity and structure. We used a combination of mitochondrial DNA sequences (mtDNA) and six nuclear microsatellite loci to address two primary topics. First, because of the 30-year gap preceding the rediscovery of the western population, observational data may reflect a reduction in the region's population (i.e., Yellow Rails were not detected because of low abundance). If so, then the reduction may be manifested as a genetic bottleneck and by a reduction in genetic diversity, the effects of which are well established (Briskie and Mackintosh 2004, Frankham 2005). Therefore, we used our genetic data to quantify patterns of genetic diversity in breeding populations of the Yellow Rail while also identifying and evaluating evidence for recent bottlenecks. Second, given the disjunctness of the western population, we characterized the genetic structure of breeding populations of the Yellow Rail in North America as a whole. Significant genetic differentiation of the western group from the remainder of the species' breeding range would suggest limited demographic connectivity and emphasize the importance of the small, remnant western population.

METHODS

SPECIMEN COLLECTION

During the breeding seasons of 2005–2008 we collected blood samples from Yellow Rails in six regions across North America (Table 1, Fig. 1). We trapped the birds by imitating territorial calls and netting them when they approached. Via brachial puncture with a 26-gauge needle, we collected ~0.3 mL of blood from each individual into a heparinized tube and preserved it for subsequent genetic analyses. All

birds were banded and released after capture. Because of the capture technique and time of year of sampling, 100% of the analyzed individuals were males.

MOLECULAR METHODS

We extracted DNA as described in Haig et al. (2004). We used polymerase chain reaction (PCR) to amplify a ~625-bp fragment of the mitochondrial cytochrome *b* (cyt *b*) gene from 107 birds (Table 1). Amplification took place in 20- μ L reactions containing 2.5 mM MgCl₂, 1 μ M of primers L14996 and H15646 (Sorenson et al. 1999, Sorenson 2003), 100 μ M of each dNTP, 1 \times PCR buffer (Perkin Elmer, Waltham, MA), and 1 unit AmpliTaq Gold DNA polymerase (Perkin Elmer). Thermal cycling included an initial 10 min denaturation at 94 °C followed by 35 cycles of 30 sec at 94 °C, 30 sec at 53 °C, and 1 min at 72 °C. A final 10-min elongation at 72 °C completed each reaction. For bidirectional sequencing of PCR products we used primers L14996 and H15646 and ABI Prism Big Dye DNA sequencing chemistry on an ABI 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA) housed at the Oregon State University Center for Genome Research and Bio-computing. After sequencing, we aligned, edited, and trimmed chromatograms to a final length of 545 bp by using the program SeqMan version 8.0.2 (DNASar, Inc., Madison, WI).

We obtained nuclear microsatellite genotypes at six loci from 116 individuals (Table 1). Primers for locus Crex11 were obtained from Gautschi et al. (2002), whereas primers for loci B106, D9, and D112 were originally designed for the California Black Rail (*Laterallus jamaicensis coturniculus*; Molecular Ecology Resources Primer Development Consortium et al. 2009). We developed primers for loci YERA9 and YERA20 from Yellow Rail DNA sequenced with an Illumina 1G genome analyzer (Illumina, Inc., San Diego). Primer sequences, repeat motifs, and annealing temperatures are provided in Table 2. PCR took place in 20- μ L reactions containing 1 \times PCR buffer (Promega, Inc.), 0.5 μ M of each primer, 2.5 mM MgCl₂, 100 μ M of each dNTP, and 1 unit Taq DNA polymerase (Promega, Inc., Madison, WI). Thermal cycling entailed 3 min denaturation at 94 °C followed by 35 cycles of 30 sec at 94 °C, 30 sec at the annealing temperature specified in Table 2, and elongation at 72 °C for 1 min. A final 10-min elongation completed each reaction. Amplification products were analyzed on an ABI 3100 capillary DNA automated sequencer. We used ABI Genescan analysis software to size fragments with reference to internal lane standard GeneScan 500 (Rox). We scored allele sizes with ABI Genotyper analysis software.

STATISTICAL ANALYSES

Quantifying genetic diversity and testing for recent bottlenecks. We used the computer program Arlequin, version 3.1 (Excoffier et al. 2005), to quantify gene diversity (*H*), nucleotide diversity (π), and allelic richness (*A*) for our mtDNA data within each region sampled. Likewise, we quantified the microsatellites' genetic diversity as average observed and

TABLE 1. Sample sizes and locality information for Yellow Rails sampled in six regions.

Region and locality	Longitude	Latitude	<i>n</i> (microsatellite)	<i>n</i> (mtDNA)
Klamath Co., Oregon (OR)			28	27
Fourmile Creek	-122.06	42.62	6	6
Sycan Marsh	-121.13	42.78	5	4
Klamath Marsh National Wildlife Refuge	-121.67	42.92	17	17
Manitoba (MB)			9	9
Douglas Marsh, Westman Co.	-99.63	49.81	8	8
Douglas Marsh, Westman Co.	-99.55	49.81	1	1
Northern Minnesota/eastern Manitoba (N-MN/E-MB)			24	19
Roseau Wildlife Mgmt. Area, Roseau Co., MN	-95.96	48.98	19	15
Brokenhead Swamp, Eastman Co., MB	-96.35	49.74	5	4
Wisconsin/eastern Minnesota (WI/E-MN)			17	15
McGregor Marsh, East, Aitkin Co., MN	-93.25	46.90	12	11
McGregor Marsh, South, Aitkin Co., MN	-93.57	46.36	1	0
Crex Meadows Wildlife Area, Burnett Co., WI	-92.65	45.87	3	3
Crex Meadows Refuge Ext., Burnett, Co., WI	-92.68	45.80	1	1
Michigan (MI)			16	16
Seney Natl. Wildlife Refuge, Schoolcraft Co.	-85.97	46.26	1	1
Seney Natl. Wildlife Refuge, Schoolcraft Co.	-85.97	46.29	15	15
Quebec (QB)			22	21
Cap Tourmente, Capitale-Nationale	-70.77	47.08	1	1
Ile aux Grues, Chaudière-Appalaches	-70.53	47.08	17	16
Baie-Saint-Paul, Charlevoix	-70.50	47.44	1	1
Cacouna, Bas-Saint-Laurent	-69.51	47.92	2	2
Gaspé, Gaspésie-Îles-de-la-Madeleine	-64.47	48.83	1	1

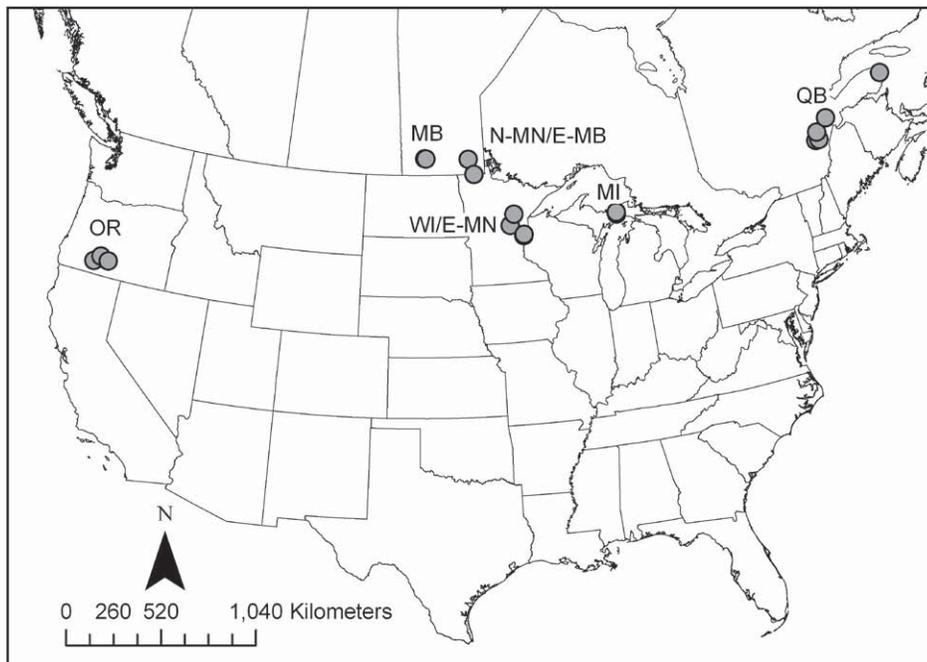


FIGURE 1. Locations of Yellow Rail sampling listed in Table 1.

TABLE 2. Primer sequences, repeat motifs, and PCR conditions for six microsatellite loci used in genetic analyses of the Yellow Rail.

Locus	Primer sequences	Repeat motif	Annealing temperature
B106	5'-CTCTTCCAGAAGCTGTAGTTG-3' 5'-TAGTGCTCTCAGGAAAGACTTG-3'	CT	53 °C
D9	5'-GCATCTTAACTGGTGTCTTG-3' 5'-CTGCTCGATTCTTCATTGAG-3'	GATA	53 °C
D112	5'-GGCTGCTCACAACTGTATC-3' 5'-TTGGATTTTTAGCCTGTC-3'	GATA	53 °C
Crex11	5'-CACCTGGTCAAGTAAGCAACC-3' 5'-GCTTGCATAACCTGTGCTTG-3'	CA	58 °C
YERA9	5'-AGGATATTATTGCGCTGA-3' 5'-ACATCTTAGTCAGTCTGGAGT-3'	CA	50 °C
YERA20	5'-AGGAAGTTTATTACACACACA-3' 5'-CAACTCATACCATTCTGTAAT-3'	CA	50 °C

expected heterozygosity over loci (H_O and H_E , respectively) and allelic richness with the computer program GDA version 1.1 (Lewis and Zaykin 2002). We also used the program HP-Rare (Kalinowski et al. 2005) to estimate allelic richness by rarefaction, and to account for differences in sample size among regions (Table 1). For our microsatellite data, we also used GDA to identify deviations from Hardy–Weinberg genotypic proportions and to test for linkage disequilibrium between pairs of loci within each region. We obtained composite test results for Hardy–Weinberg disequilibrium within each region by combining P -values from locus-specific analyses with the Z -transform test (Whitlock 2005). For our mtDNA data, we generated a haplotype network in TCS version 1.21 (Clement et al. 2000) to visualize the genealogy and level of diversity among the mitochondrial haplotypes.

We also used our microsatellites to evaluate evidence for recent population bottlenecks within each region by using the program BOTTLENECK version 1.2.02 (Piry et al. 1999). Initially, we ran analyses by assuming that the nuclear loci evolved according to either a strict stepwise mutational model or the infinite-alleles model. Neither of these models, however, appropriately accounts for mutational dynamics at microsatellite loci. Instead, a hybrid model (the two-phase model, Di Rienzo et al. 1994) appears to be much better suited. The two-phase model requires specification of two parameters: (1) the percentage of mutations that follow a strict stepwise mutational process and (2) the variance in size of multistep mutations. Therefore, we also ran our bottleneck analyses under the two-phase model using a range of parameter values. Emerging insights into the mutational dynamics of avian microsatellites suggest that only ~60% to 80% of mutations involve a single-step change (Brohede et al. 2002, 2004, Beck et al. 2003, Ibarguchi et al. 2004, Ortego et al. 2008). Consequently, in an attempt to bracket the values observed in empirical data sets, we ran our two-phase-model analyses using values of either 60% or 80% pure stepwise mutations. Likewise, on the basis of the observed ranges

of microsatellite allele sizes detected in Yellow Rails (Table 3), we specified the variances of multistate mutational sizes as 4, 9, 16, or 25, which correspond to average multistate mutational jumps of ~2, 3, 4, or 5 steps (Di Rienzo et al. 1994). In all bottleneck analyses we used 10 000 replicates, with results over loci derived from the Wilcoxon signed-rank test as suggested by Cornuet and Luikart (1996). To complement these simulation-based analyses, we also collated evidence for the presence of skewed distributions of allele frequencies within each region sampled (Luikart et al. 1998). Although this approach does not constitute a formal statistical test, detection of these pattern types can also provide heuristic evidence for a recent bottleneck (Luikart et al. 1998).

Genetic structure patterns. We used several different approaches to identify genetic structure among Yellow Rail populations. First, we used STRUCTURE version 2.2.3 (Pritchard et al. 2000), a Bayesian clustering program, to identify the number of genetic clusters suggested by the microsatellite loci and to assign each analyzed individual to one of the identified clusters. These analyses, in which we assumed numbers of clusters (K) ranging from one through six, had an initial 2×10^5 burn-in steps followed by 3×10^6 analysis replicates. As suggested by the program's authors (Falush et al. 2003), analysis options included the correlated allele frequency model and the admixture model. We ran 10 replicate analyses for each value of K and summarized results from values of K that yielded the highest average likelihood score with the computer program CLUMPP version 1.1.1 (Jakobsson and Rosenberg 2007).

Second, we used Arlequin for analyses of molecular variance (AMOVA; Excoffier et al. 1992), to calculate F -statistics, and to quantify genetic structure patterns. For mtDNA, Φ_{ST} was calculated by using the distance matrix of pairwise nucleotide-substitution differences between haplotypes. For the microsatellites, we estimated F_{ST} by (1) ignoring size differences among microsatellite alleles, which results in values of F_{ST} equivalent to estimates of θ as outlined by Weir and

TABLE 3. Allele frequencies at six Yellow Rail microsatellite loci within the six regions sampled. See Table 1 for abbreviations.

Locus	Allele size	Region						Overall
		OR	QB	MB	MI	N-MN/ E-MB	WI/E-MN	
B106	155	0.143	0.091	0.111	0.094	0.188	0.088	0.125
	157	0.000	0.000	0.000	0.000	0.021	0.000	0.004
	159	0.107	0.205	0.333	0.219	0.167	0.265	0.194
	161	0.000	0.023	0.000	0.000	0.000	0.059	0.013
	169	0.018	0.000	0.000	0.000	0.000	0.000	0.004
	171	0.071	0.023	0.111	0.063	0.021	0.029	0.047
	177	0.375	0.432	0.222	0.406	0.396	0.382	0.384
	179	0.107	0.205	0.111	0.219	0.188	0.177	0.168
	181	0.179	0.023	0.056	0.000	0.021	0.000	0.056
	185	0.000	0.000	0.056	0.000	0.000	0.000	0.004
D9	121	0.036	0.000	0.000	0.000	0.000	0.000	0.009
	125	0.036	0.023	0.000	0.000	0.000	0.000	0.013
	129	0.036	0.046	0.111	0.156	0.000	0.088	0.060
	137	0.000	0.000	0.000	0.000	0.021	0.029	0.009
	141	0.250	0.023	0.056	0.063	0.063	0.147	0.112
	147	0.000	0.023	0.000	0.000	0.000	0.029	0.009
	151	0.411	0.318	0.222	0.188	0.438	0.265	0.332
	155	0.000	0.068	0.056	0.000	0.021	0.000	0.022
	159	0.000	0.091	0.056	0.188	0.063	0.088	0.073
	163	0.232	0.409	0.500	0.375	0.396	0.353	0.358
D112	167	0.000	0.000	0.000	0.031	0.000	0.000	0.004
	91	0.000	0.023	0.000	0.000	0.021	0.000	0.009
	95	0.143	0.046	0.056	0.000	0.063	0.059	0.069
	99	0.143	0.046	0.000	0.125	0.021	0.000	0.065
	103	0.000	0.023	0.111	0.063	0.042	0.000	0.030
	107	0.125	0.046	0.056	0.000	0.021	0.059	0.056
	111	0.286	0.182	0.167	0.156	0.188	0.235	0.211
	115	0.125	0.250	0.278	0.219	0.313	0.235	0.228
	119	0.161	0.159	0.111	0.188	0.125	0.206	0.160
	123	0.018	0.091	0.167	0.188	0.188	0.206	0.129
Crex11	127	0.000	0.091	0.056	0.031	0.021	0.000	0.030
	131	0.000	0.023	0.000	0.031	0.000	0.000	0.009
	135	0.000	0.023	0.000	0.000	0.000	0.000	0.004
	82	0.196	0.091	0.111	0.094	0.063	0.118	0.116
	84	0.232	0.250	0.333	0.219	0.396	0.294	0.285
	86	0.018	0.046	0.056	0.031	0.063	0.029	0.039
	88	0.179	0.159	0.167	0.125	0.146	0.088	0.147
	90	0.125	0.114	0.000	0.094	0.083	0.147	0.103
	92	0.232	0.296	0.278	0.438	0.188	0.235	0.267
	94	0.018	0.046	0.056	0.000	0.063	0.088	0.043
YERA9	89	0.089	0.250	0.167	0.125	0.146	0.177	0.155
	91	0.000	0.068	0.000	0.063	0.083	0.029	0.043
	93	0.536	0.523	0.389	0.531	0.479	0.471	0.500
	95	0.375	0.159	0.444	0.281	0.292	0.324	0.302
YERA20	92	0.054	0.000	0.000	0.031	0.000	0.000	0.017
	94	0.036	0.000	0.000	0.000	0.000	0.000	0.009
	96	0.196	0.432	0.333	0.281	0.271	0.265	0.289
	98	0.714	0.546	0.611	0.688	0.688	0.677	0.660
	100	0.000	0.023	0.056	0.000	0.042	0.059	0.026

Cockerham (1984), and (2) accounting for size differences among alleles, which results in F_{ST} estimates that are analogs of Slatkin's (1995) R_{ST} . We obtained P -values associated with F -statistics by a procedure based on 10 000 randomization replicates. Likewise, we obtained pairwise F_{ST} values (and associated P -values) for all pairwise combinations of the regions sampled by the three approaches outlined above.

Finally, we tested for the signature of isolation-by-distance patterns within our data sets by examining correlations between geographical and genetic distances of the areas sampled. We quantified geographical distances between areas as the distances between the average longitude and latitude coordinates associated with samples from each region (Table 1). We obtained genetic distance matrices from the pairwise estimates of Φ_{ST} described above, performing congruent analyses with both variants described for our microsatellite data. P -values associated with observed correlation coefficients were obtained with Mantel tests (Mantel 1967) based on 10 000 randomization replicates.

RESULTS

GENETIC DIVERSITY AND TESTS FOR RECENT BOTTLENECKS

We detected 18 unique cytochrome *b* haplotypes among the 107 Yellow Rails sequenced for this study (Table 4; Genbank accession numbers JN131518–JN131535). Nucleotides

varied at 17 segregating sites, five of which resulted in amino acid polymorphisms. Among the diversity-associated statistics we calculated with these data, ordinal ranks of point estimates were consistently lowest for Oregon and highest for Quebec (Table 5). The haplotype network generated from these sequences revealed a well-resolved genealogy characterized by relatively small differences among haplotypes (Fig. 2). Over 80% of the individuals bore one of two haplotypes shared by all six regions sampled (Fig. 3, Table 4). The remaining 16 haplotypes occurred at low frequencies within single regions. In contrast to the mitochondrial data, the microsatellites did not show consistent trends in genetic diversity by location. However, the samples from Oregon produced the lowest rarefied estimate of allelic richness, the lowest value of H_o , and the second lowest value of H_e (Table 5). Tests for deviations from Hardy–Weinberg genotypic proportions revealed no significant tests after sequential Bonferroni corrections. Likewise, the 90 linkage disequilibrium tests performed (15 locus pairs per population \times 6 populations) revealed only one significant outcome at the $\alpha = 0.05$ level, a result that could have been observed by chance alone.

In bottleneck analyses, we found limited evidence for recent reductions in population size in analyses using the stepwise mutational model. However, evidence from other mutational models suggested bottlenecks within the Oregon, Michigan, and Wisconsin/Minnesota regions.

TABLE 4. Absolute and relative (in parentheses) frequencies of 18 cytochrome *b* haplotypes of the Yellow Rail within the six regions sampled. See Table 1 for abbreviations.

Haplotype	Region						Total
	MB	MI	N-MN/ E-MB	WI/E-MN	OR	QB	
H1	6 (0.667)	11 (0.688)	9 (0.474)	9 (0.600)	22 (0.815)	7 (0.333)	64 (0.598)
H2	1 (0.111)						1 (0.009)
H3	1 (0.111)	3 (0.188)	6 (0.316)	2 (0.133)	2 (0.074)	8 (0.381)	22 (0.206)
H4	1 (0.111)						1 (0.009)
H5		1 (0.063)					1 (0.009)
H6		1 (0.063)					1 (0.009)
H7				2 (0.133)			2 (0.019)
H8				1 (0.067)			1 (0.009)
H9				1 (0.067)			1 (0.009)
H10			2 (0.105)				2 (0.019)
H11			1 (0.053)				1 (0.009)
H12			1 (0.053)				1 (0.009)
H13					2 (0.074)		2 (0.019)
H14					1 (0.037)		1 (0.009)
H15						2 (0.095)	2 (0.019)
H16						1 (0.048)	1 (0.009)
H17						2 (0.095)	2 (0.019)
H18						1 (0.048)	1 (0.009)
Total	9	16	19	15	27	21	107

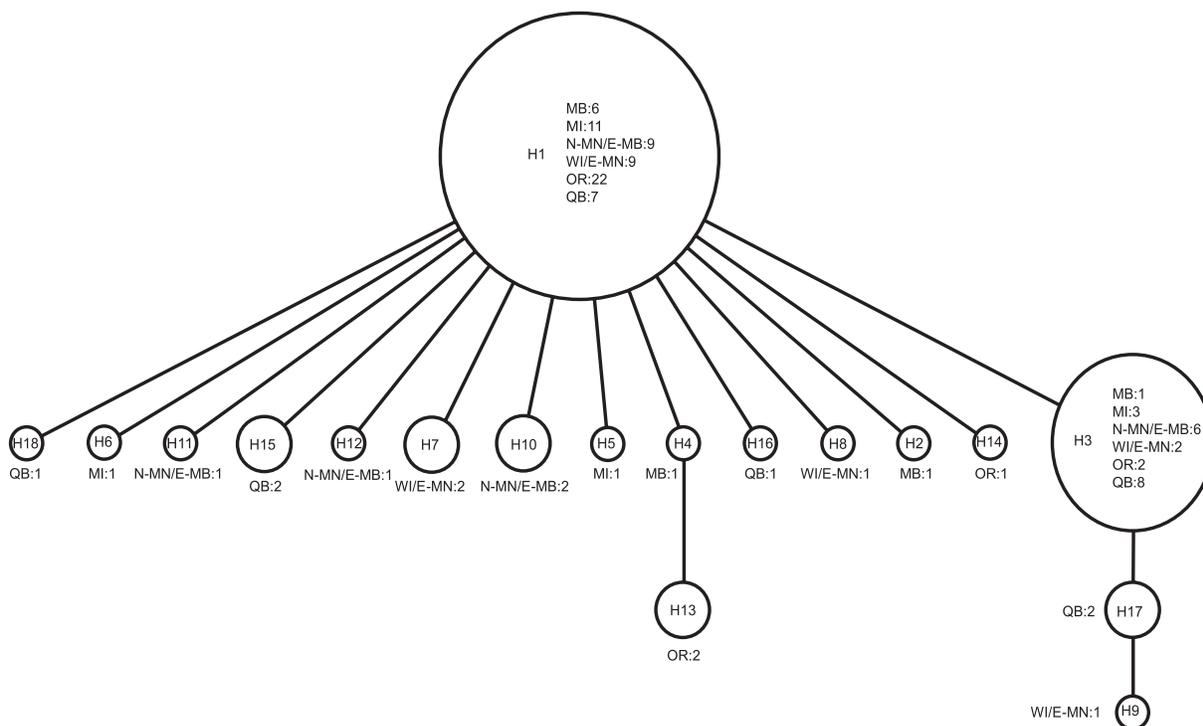


FIGURE 2. Haplotype network reflecting the genealogy of 18 mitochondrial DNA haplotypes detected in this study of the Yellow Rail. Circle sizes reflect the overall frequency of each haplotype in the data set (Table 4). Numbers after the abbreviation for each region sampled (see Table 1) are counts of each haplotype within that region (see also Table 4).

With one exception, all analyses using the infinite-alleles and two-phase models for these regions produced results significant at the $\alpha = 0.05$ level (Table 6). Although this general pattern did not hold for Oregon samples when analyzed with 80% strict stepwise mutations and a two-phase-model variance of 4, the result was nonetheless approximately significant ($P = 0.078$; Table 6). Furthermore, Oregon was the only region sampled where we

detected a mode shift in the allele-frequency spectrum (Table 6), a finding that is also consistent with a recent bottleneck at that location.

GENETIC STRUCTURE PATTERNS

Different analyses provided varying insights regarding the Yellow Rail’s patterns of genetic structure. For example, STRUC-TURE analyses provided no evidence of genetic structure.

TABLE 5. Genetic diversity^a of microsatellites and mitochondrial cytochrome *b* sequences in samples of the Yellow Rail from six regions.

Region	Microsatellite				mtDNA			
	<i>n</i>	<i>A</i>	<i>H_E</i>	<i>H_O</i>	<i>n</i>	<i>A</i>	<i>H</i>	π
OR	28	5.67 (4.72)	0.700	0.649	27	4 (2.46)	0.336	0.0009
MB	9	5.50 (5.50)	0.743	0.704	9	4 (4.00)	0.583	0.0012
N-MN/E-MB	24	6.17 (4.79)	0.693	0.722	19	5 (3.68)	0.696	0.0016
WI/E-MN	17	5.50 (4.87)	0.725	0.755	15	5 (3.91)	0.638	0.0018
MI	16	5.33 (4.79)	0.708	0.760	16	4 (3.06)	0.517	0.0011
QB	22	6.83 (5.23)	0.723	0.689	21	6 (4.22)	0.757	0.0019

^a*A*, allelic richness (with rarefied estimates accounting for differences in sample size in parentheses), *H_E*: expected heterozygosity, *H_O*: observed heterozygosity, *H*: mitochondrial gene diversity, π : nucleotide diversity.

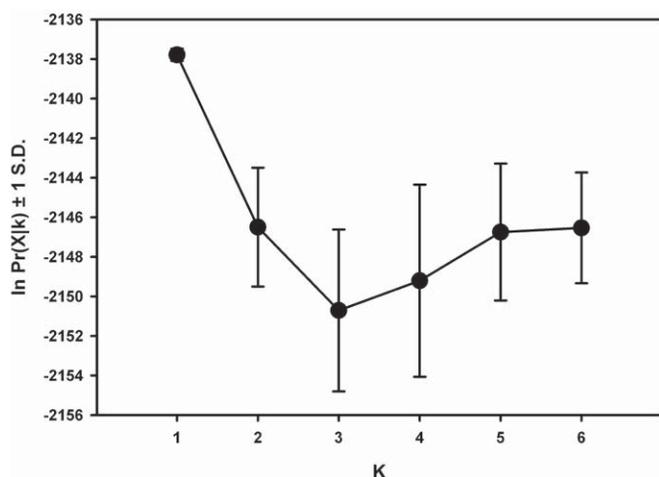


FIGURE 3. Results of STRUCTURE analyses of the microsatellite data. Average likelihood scores were lowest for the case of $K = 1$, suggesting the presence of only one genetic cluster.

Among the values of K investigated, the greatest average likelihood score was for the case of $K = 1$ (Fig. 3), suggesting that there is no population subdivision within the species. However, other analyses conflicted with this result. The global estimate of F_{ST} derived from the mtDNA indicated significant genetic structure ($F_{ST} = 0.051, P = 0.010$) and that the differentiation involved mainly a few pairwise contrasts between regions (Table 7). Furthermore, despite the microsatellite data yielding a nonsignificant global estimate of θ ($0.005, P = 0.995$), three of the five pairwise contrasts involving Oregon revealed significant differences at the $\alpha = 0.05$ level (Table 7). Values of θ from pairwise contrasts involving Oregon were large relative to those involving locations within the Yellow Rail's main breeding range. Nine of the 10 pairwise contrasts from the main breeding range yielded negative values of θ (Table 7). Consistent with results of mtDNA analyses, R_{ST} estimates derived from the microsatellite data

revealed highly significant overall evidence for genetic structure ($R_{ST} = 0.038, P = 0.009$). Results from pairwise contrasts of regions showed that the differentiation was due solely to Oregon, which was markedly differentiated from all five of the other regions sampled (Table 7) when the R_{ST} statistic was used. Like the pairwise θ estimates, values of R_{ST} produced in contrasts involving Oregon were substantially greater than those observed within the Yellow Rail's main breeding population (Table 7).

Our Mantel test results also suggested that genetic structure generally took the form of isolation-by-distance patterns. In analyses of the mtDNA, evidence suggested the presence of a correlation between geographic distance and pairwise F_{ST} values ($r = 0.690, P = 0.049$). We observed stronger patterns in the microsatellite data ($\theta: r = 0.789, P = 0.013; R_{ST}: r = 0.732, P = 0.056$).

DISCUSSION

GENETIC STRUCTURE

The western population of the Yellow Rail is enigmatic. It is situated far from the majority of the species' breeding range (Fig. 1), and the degree of demographic connectivity between populations from these two regions is currently unknown. In the United States, the distribution of the Yellow Rail's main breeding population is believed to reach its westernmost limits in northeastern Montana (Bookhout 1995). The scattered reports from Idaho (Taylor and Trost 1987, Trochlell 2005), Colorado (Griese et al. 1980), Nevada (Linsdale 1951), and Washington (Furrer 1974) are thought to represent migrants only. It is not known if these observations are of vagrants that strayed from typical migratory pathways between the winter and breeding ranges or if they reflect exchange between the western and the main breeding population. However, of the >400 male Yellow Rails banded in Oregon from 1995 to 2005, none have been recaptured in eastern North America where the species is being actively studied and banded (K. Popper,

TABLE 6. P -values from analyses designed to identify evidence for recent bottlenecks^a within the six regions where Yellow Rails were sampled. Significant results ($P < 0.05$) are highlighted in italics.

Region	TPM										
	IAM	(4-60)	(4-80)	(9-60)	(9-80)	(16-60)	(16-80)	(25-60)	(25-80)	SMM	Mode shift?
OR	<i>0.016</i>	<i>0.039</i>	0.078	<i>0.023</i>	<i>0.039</i>	<i>0.023</i>	<i>0.039</i>	<i>0.023</i>	<i>0.039</i>	0.344	Y
MB	<i>0.016</i>	0.219	0.219	0.078	0.219	0.078	0.219	0.078	0.219	0.422	N
N-MN/E-MB	<i>0.008</i>	0.422	0.656	0.281	0.500	0.078	0.422	0.078	0.344	0.961	N
WI/E-MN	<i>0.008</i>	0.219	N								
MI	<i>0.008</i>	<i>0.008</i>	<i>0.023</i>	<i>0.008</i>	<i>0.016</i>	<i>0.008</i>	<i>0.016</i>	<i>0.008</i>	<i>0.016</i>	0.078	N
QB	<i>0.016</i>	0.422	0.656	0.422	0.422	0.344	0.422	0.281	0.422	0.922	N

^aIAM: infinite-alleles model, TPM: two-phase model, SMM: stepwise-mutational model. Under results from TPM analyses, values in parentheses reflect parameter settings employed. The first value reflects the variance in the size of nonstepwise mutations, the second reflects the percent of mutations that adhered to a strict stepwise mutational model.

TABLE 7. Global (in first column) and pairwise estimates of F_{ST} for regional populations of the Yellow Rail. Values of F_{ST} are below and P -values are above matrix diagonals. Significant contrasts ($P < 0.05$) are highlighted in italics solely for the purposes of illustrating general trends.

	MB	MI	N-MN/ E-MB	WI/E-MN	QB	OR
Mitochondrial DNA: $\Phi_{ST} = 0.052$, $P = 0.015$						
MB	*	0.604	0.261	0.560	0.617	0.884
MI	-0.024	*	0.343	0.553	<i>0.047</i>	0.281
N-MN/E-MB	0.021	-0.002	*	0.288	0.212	<i>0.017</i>
WI/E-MN	-0.016	-0.014	0.007	*	0.095	0.121
QB	0.098	<i>0.072</i>	0.016	0.053	*	<i>0.000</i>
OR	-0.035	0.013	<i>0.082</i>	0.035	<i>0.180</i>	*
Microsatellite results by Weir and Cockerham's (1984) approach: $\theta = 0.005$, $P = 0.995$						
MB	*	0.812	0.797	0.975	0.758	0.104
MI	-0.013	*	0.207	0.925	0.737	<i>0.013</i>
N-MN/E-MB	-0.011	0.005	*	0.929	0.548	<i>0.012</i>
WI/E-MN	-0.022	-0.013	-0.011	*	0.790	0.166
QB	-0.008	-0.006	-0.002	-0.007	*	<i>0.004</i>
OR	0.018	<i>0.022</i>	<i>0.017</i>	0.008	<i>0.027</i>	*
Microsatellite analyses incorporating variation in allele sizes (sensu Slatkin 1995). See text for more details. $R_{ST} = 0.038$, $P = 0.011$						
MB	*	0.825	0.986	0.808	0.806	<i>0.020</i>
MI	-0.031	*	0.602	0.855	0.913	<i>0.006</i>
N-MN/E-MB	-0.036	-0.012	*	0.352	0.762	<i>0.001</i>
WI/E-MN	-0.028	-0.023	0.000	*	0.445	<i>0.005</i>
QB	-0.025	-0.022	-0.013	-0.004	*	<i>0.001</i>
OR	<i>0.083</i>	<i>0.075</i>	<i>0.113</i>	<i>0.073</i>	<i>0.107</i>	*

personal communication). In most cases, investigations that have successfully monitored movements of individually marked Yellow Rails have focused on estimating small-scale home-range sizes or local dispersal patterns (Bookhout and Stenzel 1987, Robert and Laporte 1999; K. Popper, unpubl. data) rather than following birds throughout the annual cycle. To our knowledge, there have been no formal attempts to track individual Yellow Rails during long-distance migration or dispersal. Such an endeavor could be formidable given the Yellow Rail's elusiveness and the difficulty of capturing it in appreciable numbers (Robert and Laporte 1997).

Our results allowed us to tentatively identify the degree of isolation of the Oregon population. With the exception of our STRUCTURE analyses (Fig. 3), they indicated significant genetic structure within the Yellow Rail among the regions sampled (Table 7). The discrepancy between approaches can be attributed to STRUCTURE's inability to detect weak genetic structure or patterns of isolation by distance (see sections 4.4 and 4.5 of STRUCTURE's documentation; Latch et al. 2006, Schwartz and McKelvey 2009), both of which were evident in our data set (Table 7, see Results). Thus a more complete understanding of the Yellow Rail's patterns of genetic structure was probably revealed by our F -statistics analyses (Table 7) and Mantel tests.

Analyses of the microsatellite data generally indicated that the Oregon population was significantly differentiated from populations of the remaining five regions sampled (Table 7). In general, pairwise θ and R_{ST} values obtained from contrasts involving the Oregon population were far greater than values obtained from contrasts involving pairs of regions within the Yellow Rail's main breeding range (Table 7). Nonetheless, the differentiation of the Oregon population was subtle and primarily reflected regional differences in allele or haplotype frequencies (Tables 3, 4, 7). Avise and Walker's (1998) reanalysis of data from 63 avian species implicated the effects of Pleistocene climatic cycles on genetic structure patterns within species. Given the high degree of haplotype sharing and low level of divergence among populations (Fig. 2), it remains plausible that the split between the Oregon and main breeding populations of the Yellow Rail is relatively recent, also coinciding with Pleistocene climate changes. Likewise, this result may indicate that the physical isolation of the western population also reflects reduced demographic connectivity of the western and main populations. Because we detected significant correlation between genetic and geographic distances, the large geographic distance between the western and main breeding populations may be the sole basis for this pattern.

Genetic structure patterns identified by the mtDNA were not as clear as those identified by the microsatellites (Table 7). However, the global value of F_{ST} for the mtDNA was greater than the indicators of differentiation generated for the microsatellite data set, as predicted by population genetic theory (Larsson et al. 2009). Although the number of individuals represented in the nuclear and mtDNA data sets is similar, the mtDNA data set contains half the number of alleles of the microsatellite data set because of the mitochondrial genome's haploid structure. Consequently, the power of analyses to detect subtle but significant structure with the mtDNA may have been reduced relative to the microsatellite data because fewer alleles were effectively available for estimation and analysis of allele frequencies. It is also possible that our mtDNA results were influenced by our sample being biased toward males. We note, however, that this influence will become apparent only under limited circumstances. Because mtDNA is maternally inherited, patterns of genetic structure can reflect dispersal and movement tendencies of females. If dispersal patterns are similar in both sexes, then observed differentiation at mtDNA loci from a sample of males will not be affected and will provide an accurate reflection of divergence in mitochondrial DNA. If females disperse more than males the outcome will be similar. In this case, because males are relatively sedentary and do not transmit mtDNA to their offspring, they will display the mtDNA structure patterns generated by their female parents in prior generations. The only situation in which male-biased specimen sampling influences observed mtDNA genetic structure is when males disperse more than females. In this scenario, if only males are included in genetic analyses, mtDNA differentiation will underestimate the true degree of structure at mtDNA loci—a situation likely not applicable to this investigation. Studies of avian dispersal have generally shown that females' fidelity to a breeding site is less than that of males (Greenwood and Harvey 1982). If this pattern holds for the Yellow Rail, then mtDNA genetic structure observed in a sample of males will accurately reflect females' patterns of mtDNA for the reasons noted above.

The levels of differentiation we observed among Yellow Rail populations across North America were similar to or less than the differentiation seen among geographically closer populations of the Black Rail (*Laterallus jamaicensis*) and Clapper Rail (*Rallus longirostris*) analyzed from California alone (Girard et al. 2010, Fleischer et al. 1995). Thus Yellow Rails may disperse more readily than either Black Rails or Clapper Rails. Additional empirical data on movement of all of these species are needed to verify such behavioral differences.

GENETIC BOTTLENECKS AND DIVERSITY

In addition to identifying genetic structure within the Yellow Rail, we also found evidence for recent genetic bottlenecks in three of the six regions sampled (Oregon, Michigan, and Wisconsin/eastern Minnesota; Table 6). We do not know if this high percentage is representative of the Yellow Rail's entire

range or if we happened to encounter multiple bottlenecks by chance alone. Future investigations that sample additional regions from across the species' breeding range will be required to address this topic.

Outcomes of bottleneck analyses can be influenced by the choice of mutational models and parameters (Table 6; Williamson-Natesan 2005). Tests based on the simple step-wise mutational model never produced significant results; however, we note that empirical evaluations of the mutational dynamics of avian microsatellites suggest that the two-phase model is a much more appropriate framework for bottleneck analyses (Brohede et al. 2002, 2004, Beck et al. 2003, Ibaraguchi et al. 2004, Ortego et al. 2008). In our analyses based on the two-phase model, we used a range of realistic mutational parameters obtained directly from published avian-pedigree analyses (see Methods), suggesting that our results should be considered robust, realistic, and free of bias that could have been introduced if arbitrary parameter values were chosen.

Our analyses also revealed evidence for shifted allele-frequency distributions within the Oregon population, which provided corroborating evidence for a recent bottleneck within that region. Although we did not observe comparable patterns for Michigan and Wisconsin/eastern Minnesota, we note that samples from these two areas were substantially smaller than that from Oregon (Table 1). On the basis of computer simulations, Luikart et al. (1998) illustrated that the power of the approach of shifted allele-frequency spectrum is highly dependent on the number of individuals sampled. Thus the absence of this specific signal from Michigan and Wisconsin/eastern Minnesota may simply be an artifact of the ~40–45% smaller samples from these two populations. The ability to detect a prior population bottleneck is also influenced by the magnitude of the past reduction in population size (Cornuet and Luikart 1996, Luikart et al. 1998, Williamson-Natesan 2005), with larger population reductions following the original disturbance being more easily detected. It is possible that the Oregon population was reduced more sharply. Indeed, although our sample from Oregon was the largest of any region, our analysis of Oregon birds consistently revealed the lowest levels of genetic diversity (Table 5) and provided additional evidence consistent with a comparatively strong bottleneck within the region.

Determining the precise causes and timings of past bottlenecks can be difficult. Wetland loss is generally considered to be the greatest threat to most rail populations (Eddleman et al. 1988). Consequently, factors that influence availability of wetlands may provide important cues that help identify important causal factors and events. In Oregon, the Yellow Rail is listed as "sensitive-critical" (Oregon Department of Fish and Wildlife 2008) because of its scarcity and the long period over which it was not observed in the state. It has been estimated that 85% of wetlands in the Klamath Basin of Oregon and northern California, as well as the marshes

of San Francisco Bay, have been lost since 1900 because of development or agricultural conversion (Bottorff 1989, Stern et al. 1993, Popper and Stern 2000, Dedrick 1989). Given the Yellow Rail's strong reliance on wetlands for breeding and wintering habitat (Robert and Laporte 1997, Goldade et al. 2002), these activities may have decimated local Yellow Rail populations and account for the 30-year absence of records of the species from the region. If so, this process also likely resulted in the reduction of the Oregon population detected by our genetic-bottleneck analyses (Table 6).

Loss of wetlands in Michigan has been estimated to be at least 50% (Dahl 1997), contributing to loss of Yellow Rail habitat around Seney National Wildlife Refuge (the origin of our Michigan samples). The genetic bottleneck implied by our analyses for this site may be explained not only by that direct loss of habitat but also by the additional temporary loss of habitat to drought and fire. Michigan's eastern Upper Peninsula experienced multiple multi-year droughts during the 20th century (data from NOAA, National Climatic Data Center, <http://cdo.ncdc.noaa.gov/>), and observations from 2007 to 2009 demonstrate that Yellow Rail numbers drop in drought years (Austin 2009); successive years of drought presumably have an even more severe effect on the local breeding population. This additional loss of habitat, possibly combined with additional temporary habitat loss from a large fire, such as the Seney Fire of 1976 that burned over much of the refuge (Anderson 1982, Drobyshev et al. 2008), likely affected the population negatively and caused individuals to fly farther north to breed in Canada (J. Austin and D. Olsen, pers. comm.).

At the third site where our analysis identified a genetic bottleneck, Nelson (1991) highlighted the importance of a drought from 1987 to 1989 that appeared to have negative repercussions for the Yellow Rail population at McGregor Marsh, Minnesota (the locality where most of the Wisconsin/eastern Minnesota samples originated; Table 1). Although 15 calling males at this small (~8 km²) wetland were typically observed during the 1970s and early 1980s, only two males were reported during 1987 and 1988. Given that droughts affect regions rather than specific localities, this finding suggests that there may have been a larger-scale reduction in population size that produced the bottleneck we detected in the Wisconsin/eastern Minnesota region. Although records of Yellow Rail abundance during the first half of the 20th century are lacking, it remains possible that these disturbances were of a magnitude sufficient to reduce the local population and produce the genetic bottleneck detected in our analyses.

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