Conservation Genetics (2005) 6:159–174
DOI 10.1007/s10592-004-7831-9

Population genetic structure and dispersal across a fragmented landscape in cerulean warblers (*Dendroica cerulea*)

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Received 11 May 2004; accepted 18 August 2004

Key words: cerulean warbler, conservation genetics, dispersal, habitat fragmentation, population genetic structure

Abstract

Cerulean warblers (*Dendroica cerulea*) have experienced significant declines across their breeding range and presently exist in disjunct populations, largely because of extensive loss and fragmentation of their breeding and wintering habitat. Despite this overall decline, a recent north-eastern expansion of the breeding range has been proposed, and some researchers have suggested that the eastern Ontario population may be acting as a source population maintaining sink populations elsewhere. However, little is known about either the geographic distribution of genetic variation or dispersal in these birds. We assayed variation in five microsatellite loci and a 366 base-pair fragment of the mitochondrial control region among 154 cerulean warblers from five populations throughout the breeding range. No evidence of population genetic structure was found. Assignment tests suggested that six individuals were either inter-population migrants or descendants of recent migrants. The lack of population genetic structure is probably due to a combination of historical association and contemporary dispersal. Population decline does not appear to have reduced genetic variation yet. Overall results suggest that cerulean warblers from Ontario, Illinois, Arkansas and Tennessee should be considered a single genetic management unit for conservation.

Introduction

Cerulean warblers (*Dendroica cerulea*) are experiencing the largest population decline of any North American warbler (3.04% per year between 1966 and 2000; Link and Sauer 2002), and have disappeared or decreased in areas of former abundance within the last century, particularly in the western and south-western edge of their breeding range (Hamel 2000a). These neotropical migrants currently winter along the northern Andes Mountains in northern South America and breed throughout much of eastern North America, but are limited to large patches of mature, deciduous forest for nesting (Figure 1; Hamel 2000a; Hamel et al. 2004). They are extremely sensitive to deforestation, since habitat fragmentation augments brood parasitism by brown-headed cowbirds (*Molothrus ater*) and nest predation (Robbins et al. 1992; Robinson et al. 1995). In addition, small forest fragments exhibit substantially lower prey abundance than larger fragments (Burke and Nol 1998). These factors combine to reduce the reproductive success of cerulean warblers throughout much of their range, and population models suggest that the species may not be self-sustaining (Jones et al. 2004). In Canada they are currently listed as a Species of Special Concern by COSEWIC (2000), and in the United States they have varied legal status, including Rare, Threatened, and Special

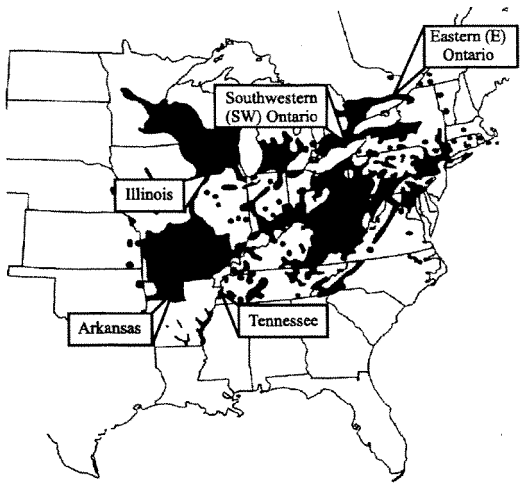

Figure 1. Current breeding range of cerulean warblers (shaded area, adapted from Dunn and Garrett 1997), and regions sampled for this study.

Management Concern (Hamel 2000b). Nonetheless, a north-eastern expansion of their breeding range has been suggested, as agricultural land abandoned at the turn of the 20th century succeeds to mature forest (Hamel 1992; Robbins et al. 1992; Sauer et al. 2003). In addition, on-going research indicates that the eastern Ontario population is experiencing high reproductive success (Oliarnyk and Robertson 1996). This peripheral, reproductively successful population could potentially act as a source, exporting surplus recruits to sink populations, where mortality exceeds reproduction (Dias 1996; Hamel et al. 2004, but see Jones et al. 2004). However, little is known about either the geographic distribution of genetic variation in cerulean warblers or the dispersal patterns or site fidelity of adults or juveniles (Hamel et al. 2004). Only 1605 cerulean warblers have been banded in North America since 1950, with only two birds ever recaptured away from their banding sites (Hamel 2000b, United States Geological Service, Patuxent Wildlife Research Center 2003). While breeding site philopatry has been documented both for males and for females (Hamel 2000b; Jones et al. 2004; Barg et al. unpubl. data), the fate of birds that do not return is uncertain. Thus, information on population genetic structure and dispersal is needed to aid the management of this species. In the present study, we used molecular markers to determine the distribution of genetic variation within and among breeding populations of cerulean warblers, and to gain insight into the demographic history and extent of contemporary dispersal within the species.

Materials and methods

Sampling

Blood was sampled from 161 cerulean warblers from one to six sites within each of five regions between May and early July, 1997–1999 (Figure 1, Table 1). Adult males were captured within breeding territories using a mistnet, conspecific song playback and a model. Two breeding females and one fledgling also were captured by passive mistnetting. Each bird was banded with a Canadian Wildlife Service or U.S. Fish and Wildlife Service band. Blood samples were obtained from the brachial vein, and stored either in 70% ethanol or desiccated on filter paper. Total genomic DNA was isolated from blood using standard protocols for protease K digestion and phenol–chloroform extraction (Friesen et al. 1997).

Laboratory analyses

Two primers external to the mitochondrial control region, ND6L16510 (5′-CCAACGACAC(C/T)GAATAAACAAACAC-3′) and 12SH1299 (5′-AACAGTAAGGTTAGGACTAAGTCTT-3′), were designed to anneal to sites that are conserved across several passerine sequences (obtained from GenBank) in the ND6 and 12S rRNA genes, respectively. These primers were used to amplify a ~1500 bp fragment of DNA that included the 3′ end of the ND6 gene, the $tRNA^{glu}$ gene, the entire control region, and the $tRNA^{phe}$ gene from two cerulean warblers. Amplifications followed standard protocols (Friesen et al. 1997) with inclusion of 0.18 mg/ml BSA (Pharmacia Biotech) and 0.1% gelatin, annealing at 55 °C, and extension for 75 s. PCR products were visualized on 1.5% agarose gels, and purified using GeneClean III® (Bio 101, Inc.) or Agarase (Boehringer Mannheim) kits according to the manufacturer's suggested protocol. All samples were sequenced manually using Thermo Sequenase Terminator® radiolabelled cy-

Table 1. Numbers of cerulean warblers sampled at different sites within five regions

Region and sites	Coordinates	Numbers
Eastern Ontario		
Queen's University Biological Station	44°34′N 76°20′W[a]	55
South–western Ontario		
LPRCA – South Walsingham Forest	42°38′N 80°33′W	6
LPRCA – Backus Woods	42°40′N 80°29′W[a]	3
LPRCA – Cultus Forest	42°39′N 80°28′W	2
LPRCA – Walsh Forest	42°45′N 80°25′W	2
LPRCA – St. Williams Forest	42°40′N 80°28′W	1
Skunk's Misery	42°45′N 81°45′W	6
Illinois		
Mississippi Palisades State Park	42°12′N90°15′W[a]	26
Arkansas		
Cass	35°44′N 93°48′W	6
Sand Gap	35°42′N 93°04′W[a]	11
Payne Ridge	35°38′N 93°03′W	1
Beindorf	35°37′N 93°02′W	6
Tennessee		
Meeman Shelby Forest State Park	35°33′N 89°87′W[a]	28
Chickasaw National Wildlife Refuge	35°74′N 89°53′W	8

[a] Latitude and longitude used in estimating geographic distances between regions.
LPRCA = Long Point Regional Conservation Areas.

cle sequencing kits (Amersham Life Science) according to the manufacturer's instructions. Sequences were scored manually and aligned using ESEE (Cabot and Beckenbach 1989). Primers specific for cerulean warblers (MCRL554: 5′-CTTGCTCTTTTGCGCTATTGGTTG- 3′; MCRH922: 5′-AATGGAATTTAAATGTTAGT TCCTAG-3′) were designed for regions where sequences of cerulean warblers and other passerines are highly similar, and were used to amplify a 366 bp fragment of Domain II (318 bp) and Domain III (48 bp) from the control region of all samples. (Although Domains I and III of the control region are generally considered more useful than Domain II in population genetics, a nuclear paralog of Domain I and length heteroplasmy in Domain III necessitated use of Domain II in this study). For the majority of samples, sequencing was conducted using the light-strand primer (MCRL554) only; if sequence was ambiguous, samples also were sequenced with the heavy-strand primer.

All samples were screened with primers for five microsatellite loci that were previously found to be polymorphic in yellow warblers (*Dendroica petechia*, Dawson et al. 1997) or Swainson's thrushes (*Catharus ustulatus*, Gibbs et al. 1999; Appendix 1). Amplifications were conducted as described in Ibarguchi et al. (2000) with annealing at 50 °C. Amplification products were resolved on 4% polyacrylamide denaturing gels. Likelihood ratio tests for deviation from Hardy-Weinberg equilibrium and tests for linkage disequilibrium were conducted using ARLEQUIN (version 1.1, Schneider et al. 1997); sequential Bonferroni corrections were applied using a table-wide significance value of 0.05 (Rice 1989).

Analyses of within-population variation

Several tests for microgeographic structure were performed to determine the validity of pooling samples collected from different sites within a region. In regions with one large sampling area (eastern Ontario and Illinois; Table 1), relatedness coefficients were estimated from microsatellite allele frequencies as described in Queller and Goodnight (1989) using KINSHIP 1.2 (Goodnight and Queller 1999), and Kimura's two-parameter

distances (Kimura 1980) were estimated from mitochondrial control region sequences using MEGA (version 1.02; Kumar et al. 1993). The geographic distance between samples was determined from site maps, and Mantel's tests (Mantel 1967) were used to test for correlations between genetic differentiation and geographic distance. Statistical significance was assessed using the ISOLDE subroutine (Slatkin 1993) in GENEPOP (version 3.1D, Raymond and Rousset 1995) with 1000 randomized permutations of the matrices. When more than one site was sampled within a region (South-western Ontario, Arkansas, and Tennessee), pairwise geographic distances between sites were determined using the distance calculator available at <http://jan.ucc.nau.edu/~cvm/latlongdist.html>, and pairwise θ, ρ, and Φ_{ST} were estimated and tested for significance by randomization (see *Analyses of population genetic structure*, below). Mantel's tests also were performed. Although the power of these tests was limited by small sample sizes, no evidence of genetic differentiation was found within any of the regions (all $P > 0.05$). The absence of microgeographic structure was also supported by results from STRUCTURE (see *Analyses of population genetic structure*, below). Samples therefore were pooled into five "populations" or "regions" for macrogeographic analyses (Table 1). In all cases, the maximum geographic distance between sampling sites within regions was less than the minimum distance between sites from different regions.

Haplotype and nucleotide diversities (Nei 1987) were calculated from control region variation for each population using ARLEQUIN. Contemporary female effective population size (N_f) was estimated using FLUCTUATE (version 1.4; Kuhner et al. 1998), which uses a maximum likelihood approach based on coalescent theory to simultaneously estimate Θ ($2N_f\mu$, where μ is the per-site per-generation mutation rate) and population growth rate (g, in $1/\mu$ generations). The transition/transversion ratio, and initial values for Θ and population growth rate were set to 3 (the observed ratio), 0.5 (the value with the highest posterior probability from a preliminary run), and 1, respectively; however, final results were robust even to large changes in these initial values. Analyses were performed both for the total sample and for individual populations using the default search strategy and three different random seeds each. The mutation rate of Domain II of the control region has not been calibrated for warblers; to calculate N_f we therefore used a rate of 5%/my, which is intermediate between 1%/my for protein coding mitochondrial genes (Lovette 2004) and 10.4%/my for Domain I of the control region of geese (Quinn 1992). A generation time of 2 years was assumed.

Analyses of population genetic structure

For the control region, Φ-statistics (Excoffier et al. 1992) were estimated using analysis of molecular variance (AMOVA) in ARLEQUIN, with statistical significance tested using 10,000 randomizations of the data. Pairwise Φ_{ST} estimates were calculated using Kimura's (1980) two-parameter distance method with a rate parameter (α) of 0.15 for the gamma correction (Marshall and Baker 1997). A Mantel's test was used to test whether broad-scale genetic variation fits an isolation by distance pattern (as for *Analyses* of *within-population variation*).

To investigate the extent of phylogeographic structuring, relationships among mtDNA haplotypes were reconstructed using the method of statistical parsimony (Templeton 1998, 2004) using TCS (version 1.13; Clement et al. 2000). Ambiguous connections ("loops") were resolved using a hierarchy of guidelines: (1) transitions are more likely to occur repeatedly than are transversions or insertions/deletions; (2) uncommon haplotypes are more likely than common haplotypes to be tips; and (3) haplotypes are most likely to occur in geographic proximity to their direct ancestor (Crandall and Templeton 1993, Damus and Friesen, unpubl.). The parsimony tree was nested following the rules of Templeton et al. (1987), and the existence of phylogeographic structure was assessed with contingency tests using the program GEODIS (Posada et al. 2000) with 10,000 randomizations of the data.

The mutation patterns for the microsatellite loci that were used in this study were not known, so two indices of population genetic structure were used: (1) θ, which is based on the infinite alleles model (Weir and Cockerham 1984), and (2) R_{ST}, which is based on the stepwise mutation model (Slatkin 1995). θ and ρ (an unbiased estimator of R_{ST}; Goodman 1997) were estimated using FSTAT and RSTCALC (version 2.2, Goodman 1997)

respectively, and were tested for significance using 1000 randomized permutations of the data. Mantel's tests were applied to both θ and ρ to test for isolation by distance.

Microsatellite variation also was analyzed using STRUCTURE (version 2.0; Pritchard et al. 2000; Pritchard and Wen 2003), which uses a Bayesian analysis to delineate populations on the basis of deviations from Hardy–Weinberg equilibrium, independent of sampling site (Pritchard et al. 2000). Analyses were performed under the admixture model, with a burn-in of 10,000 replications, 100,000 replications after the burn-in (determined initially to be sufficient for stable posterior estimates), correlated allele frequencies, and $\lambda = 1.0$; sampling location was not used as prior information. Analyses were repeated five times each for $k = 1$ to 10 subpopulations, and the most probable value of k was identified from the posterior probabilities (Pritchard and Wen 2003).

Sequential Bonferroni corrections were applied as required (Rice 1989).

Population history

Because much of the cerulean warblers' current breeding range was occupied by glaciers until ~7,000–18,000 years ago (Pielou 1991) and the species is presently declining due to anthropogenic disturbance (see Introduction), we conducted several tests for population (mutation-drift) equilibrium: (1) The distribution of pairwise differences between control region sequences of individuals (the mismatch distribution; Rogers and Harpending 1992; Rogers 1995) was examined both for the total sample and for individual populations using ARLEQUIN. Time since expansion (t) was estimated from τ using the equation $t = \tau/2\,\mu$ (where μ in this case is per gene per year; Rogers and Harpending 1992; Rogers 1995). (2) Estimates of Tajima's D (Tajima 1989a) for control region sequences were tested for deviations from 0 both for the total sample and for individual populations using ARLEQUIN. (3) The null hypothesis that population growth rate is zero was addressed by testing whether g from FLUCTUATE (Kuhner et al. 1998) was significantly different from 0: G (twice the difference between the ln likelihood at the maximum value of g and the ln likelihood at $g = 0$) was compared with the critical value of X^2 at one degree of freedom (M. Kuhner, pers. comm.). FLUCTUATE was run with empirical base frequencies, transition/transversion = 5, initial $\Theta = 0.5$, 10 short chains with a sampling increment of 20 and 200 sampling steps along the short chains, and two long chains with a sampling increment of 20 and 20,000 steps along the long chains. (4) Nested clade analysis (NCA) was used to test for a range expansion: clades in the statistical parsimony tree (see *Analyses of population genetic structure*, above) with significantly small or significantly large geographic distributions were identified by analysis of variance using GEODIS (above), and potential instances of range expansion were identified using the inference key from Templeton (1998, 2004). (5) Microsatellite variation was tested for deviations from Hardy–Weinberg expectations using a simulation approach based on coalescent theory as implemented in BOTTLENECK (version 1.2.02; Cornuet and Luikart 1996). Since the mutation model for our loci was not known, analyses were repeated using a range of combinations of infinite alleles versus stepwise mutation models. This analysis was performed both on the total sample and on individual populations. (6) Microsatellite variation was tested for divergence–drift equilibrium using a maximum likelihood method based on coalescent theory (2MOD version 0.2; Ciofi et al. 1999). The likelihood ratio (Bayes factor) was estimated from the proportion of 100,000 simulations supporting a non-equilibrium model of divergence and drift versus migration–drift equilibrium.

Contemporary dispersal

Most methods of estimating contemporary dispersal (gene flow) from molecular data assume that populations are in genetic equilibrium; however, results of the above analyses indicated that this assumption does not hold for cerulean warblers (see Results, *Population history*). We therefore used molecular assignments to identify potential migrant individuals (Berry et al. 2004). Assignments were performed on microsatellite variation using IMMANC (version 5.1, Rannala and Mountain 1997), which uses a Bayesian approach to derive the probability densities for allele frequencies, which are then used to calculate genotype probabilities for each population. Using a posterior probability ratio test and Monte Carlo

approach, each individual is tested against the probability that it is an immigrant from another sampled population based on the multilocus genotype distributions. For cerulean warblers, statistical significance was assessed with 10,000 replications per test using a rejection level (α) of 0.01 to reduce the chances of a Type I error (Rannala and Mountain 1997).

Results

Analyses of within-population variation

Continuous DNA sequence was obtained from the 3′ end of the ND6 gene, the $tRNA^{glu}$ gene, the mitochondrial control region, and the $tRNA^{phe}$ gene, with the exception of an approximately 210 bp region at the 3′ end of the control region where length heteroplasmy prevented unambiguous sequencing. Although nuclear copies of the control region have been reported in many organisms (e.g. Kidd and Friesen 1998), overall evidence supported a mitochondrial origin for this sequence: The ND6 segment had 79% sequence similarity with the corresponding region in the chicken (*Gallus gallus*; Desjardins and Morais 1990), with no frameshift mutations. The tRNA genes were similar in length to the chicken genes and showed sufficient internal complementarity to fold into conventional cloverleaf structures with appropriate anticodon sequences (as in Desjardins and Morais 1990). The nucleotide composition of the light strand of the putative control region (A = 25.1%; C = 27.8%; G = 18.0%; T = 29.0%) was similar to that found in other avian species (Baker and Marshall 1997). The conserved sequence blocks (F, D, and C boxes, and CSB-1) had high similarities (82–100%) to those of other passerines. And, the distribution of variation fit the general pattern for avian control regions (next paragraph; Baker and Marshall 1997).

Twenty-two variable sites defined 27 control region haplotypes (Table 2), with 19 polymorphic sites in Domain II and three in Domain III (GenBank Accession Numbers AY839310-AY839336). Seventeen sites involved transitions, five involved transversions, and none involved insertions or deletions. Sequence divergence between haplotypes ranged from 0.27 to 1.64%. The most common haplotype (#1) was shared by 63% of 152 individuals, and was found across all regions. Eighteen haplotypes (67%) were unique to a particular population. The highest frequency of a unique haplotype was in Tennessee, where haplotype #15 was present in three (10%) of the 29 samples. South-western Ontario had no unique haplotypes, and shared all of its haplotypes (other than haplotype #1) with either Illinois or eastern Ontario. Haplotype and nucleotide diversities did not differ significantly among regions (ANOVA, $P > 0.10$), averaging 0.60 (SE = 0.05) and 0.26% (SE = 0.20%), respectively (Table 2). The estimate of Θ derived by FLUCTUATE (0.48, SD = 0.026) suggests a contemporary effective population size of 2.4×10^6 females.

Between eight and 24 alleles were found within each of five microsatellite loci among 154 cerulean warblers (Appendix 1). Across all loci, 45% of alleles (35 of 78) were shared among all populations. Private alleles (12% of 78) were found within all loci and all populations, but were present at low frequencies (≤4.2%). Single-locus estimates of expected heterozygosities within populations ranged from 0.30 to 0.95, with no significant differences among populations and an overall heterozygosity across all samples and all loci of 0.77 (Table 3). Dpμ 15 had a significant excess of homozygotes both for the total sample and within each population (Appendix 1), suggesting the existence of a null allele. Analyses of population structure were therefore conducted both with and without this locus to ensure that it did not bias the results. Otherwise, no evidence was found either for deviations from Hardy–Weinberg ratios or for linkage disequilibrium either overall or within populations after Bonferroni corrections (all $P > 0.08$, Appendix 1).

Analyses of population genetic structure

The global estimate of Φ_{ST} for the control region had a marginal level of statistical significance ($\Phi_{ST} = 0.01$, $P = 0.05$), suggesting weak population structure. Pairwise estimates of Φ_{ST} ranged from −0.013 to 0.051, but none were significant after Bonferroni corrections (Table 4). The statistical parsimony tree for the control region haplotypes was distinctly star-like (Figure 2). Pairwise estimates of genetic differentiation were not correlated with geographic distance between popula-

Table 2. Frequencies of control region haplotypes within five populations of cerulean warblers, sequence differences among haplotypes, and estimates of haplotypic diversity ($h \pm$ SE), nucleotide diversity ($\pm$SE) and Tajima's *D*. Identity with haplotype #1 is indicated by dots. Nucleotides are numbered relative to the 3′ end of the light strand primer. Domains are identified by comparison with *Fringilla* sequence in Baker and Marshall (1997)

Haplotype		Population[a]					Nucleotide number: Domain II 11111222222 1122236711146013466 6701419201539089156	III 333 256 193
	n	EO	SO	IL	AR	TN		
#1	96	0.64	0.80	0.65	0.50	0.59	AACTTCACGACGTGTCAGG	CAT
#2	1	0.02					.T..................	...
#3	3	0.04				0.03	..........T......A.	...
#4	5	0.06				0.07	..................A	...
#5	1	0.05					........T.T.C....A.	...
#6	8	0.06			0.15	0.03	..................G..	...
#7	3	0.05		0.08			.......T...........	...
#8	2	0.04					.......T...........	T..
#9	6	0.06	0.05		0.04	0.03	G..................	...
#10	1	0.05					..........A........	...
#11	1	0.05					.........TT......A.	...
#12	4	0.05	0.05	0.08			.....T.............	...
#13	3		0.10	0.04			..........T........	...
#14	3				0.04	0.07	..................A.	...
#15	3					0.10	...C...............	...
#16	1					0.03	..............T...	...
#17	1					0.03	..............T...	..C
#18	1			0.04			....C..............	...
#19	1			0.04			..........T...C..A.	...
#20	1			0.04			......T............	...
#21	1			0.04			..T................	...
#22	1				0.04		..........A....G..	...
#23	1				0.04		............C.....	...
#24	1				0.04		....C..........G..	...
#25	1				0.04		....	.G.
#26	1				0.04		.......T..T........	..C
#27	1				0.04		....	..C
Total	152	53	20	26	24	29		
h	0.60	058	0.36	0.58	0.74	0.65		
SE	0.05	0.08	0.13	0.11	0.09	0.10		
	0.26	0.29	0.11	0.24	0.35	0.26		
SE	0.20	0.22	0.12	0.19	0.25	0.21		
D[a]	**−2.13**	**−1.91**	**−1.44**	**−1.90**	**−1.82**	−1.67		

EO = eastern Ontario; SO = south-western Ontario; IL = Illinois; AR = Arkansas; TN = Tennessee.
[a] Tajima's *D*. Values in bold are significantly <0 at $\alpha = 0.05$.

tions (Mantel's test, $P > 0.15$). NCA indicated weak phylogeographic structure, with two of seven nesting clades having non-random distributions of haplotypes or nested clades (Appendix 2).

No global population genetic structure was evident from microsatellite variation (all estimates of θ and $\rho = 0.00$ both with and without Dpμ 15, all $P > 0.40$); pairwise estimates of θ and ρ also

Table 3. Number of individuals analyzed (N_S), and means across loci of number of alleles per locus (N_A), allelic richness (R), observed heterosygosity (H_O) and expected heterozygosity (H_E)

	E. Ontario	SW. Ontario	Illinois	Arkansas	Tennessee	Overall
N_S	55	20	26	24	29	154
N_A	14.0	9.8	11.6	11.0	11.4	15.6
R	10.5	9.8	10.6	10.5	10.5	10.4
H_O	0.65	0.70	0.69	0.62	0.73	0.68
H_E	0.76	0.77	0.75	0.76	0.81	0.77

Table 4. Pairwise estimates of Φ_{ST} (above diagonal) and geographic distance (km; below diagonal) between cerulean warbler populations

	E. Ontario	SW. Ontario	Illinois	Arkansas	Tennessee
E. Ontario	–	−0.013	−0.004	0.025	0.001
SW. Ontario	340	–	−0.019	0.051	0.013
Illinois	1150	800	–	0.046	0.017
Arkansas	1730	1330	760	–	0.039
Tennessee	1560	1770	750	240	–

gave no evidence of population differentiation (all $P > 0.05$). Neither index showed an isolation-by-distance effect (Mantel's tests, all $P > 0.15$). Results from STRUCTURE provided strong support for a single genetic population (Pr [k = 1] > 0.9999).

Population history

The mismatch distributions for control region sequences were unimodal (not shown) and did not differ from the distributions expected under a "sudden expansion" model either for the total sample or for individual populations (all $P > 0.20$). The estimate of τ for the total sample (0.92, 95% CI = 0.50–1.15) suggests that the population began to expand ~13,000 years ago (95% CI = 6800–16,000 years ago). All estimates of Tajima's D were negative, and estimates were significantly different from 0 both overall and for three populations (Table 2). Results from FLUCTUATE indicated a growth factor (g) of 860 (SD = 24.3), which is significantly >0 ($G = 133$, $P < 0.001$). NCA results for the total cladogram indicated a contiguous range expansion (Appendix 2).

None of the analyses of microsatellite variation using BOTTLENECK provided evidence of a recent change in population numbers, either for the total sample or for individual populations (all $P > 0.50$). 2MOD was unable to discriminate between a model of migration–drift equilibrium versus recent population divergence (Bayes factor = 1.01, $P = 0.50$).

Contemporary dispersal

Power on assignment tests (the probability of accepting the null hypothesis [that an individual is a resident] when the null hypothesis is false [the individual is actually an immigrant]) was between 0.70 and 0.95 for most population pairs, although power to detect migrants between eastern Ontario and Illinois was only 0.54 (Table 5). IMMANC detected six recent migrants among 154 individuals, most (5) being immigrants into eastern Ontario (Table 5).

Discussion

Population genetic structure and history

This study detected little evidence of population genetic structure in cerulean warblers, despite sampling at the northern and southern ends of

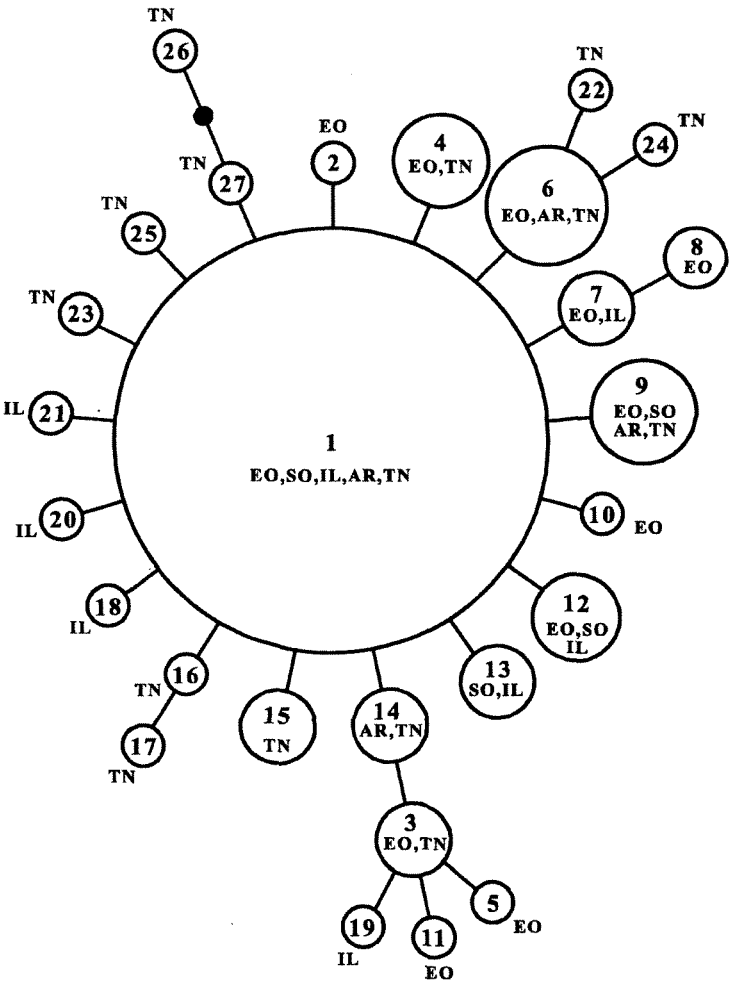

Figure 2. Statistical parsimony tree for mitochondrial control region haplotypes of cerulean warblers, and regions where haplotypes were found. Circle sizes are proportional to haplotype frequencies (Table 2). Black dot indicates a missing haplotype (one that either was not recovered during sampling, or is extinct). AR = Arkansas; EO = Eastern Ontario; IL = Illinois; SO = South-western Ontario; TN = Tennessee.

Table 5. Probability of detecting migrant individuals using the present data for cerulean warblers, and number of immigrants detected (in parentheses)

Immigrated from	Individual sampled from				
	E. Ontario	SW. Ontario	Illinois	Arkansas	Tennessee
E. Ontario	–	0.85	0.56	0.70	0.72
SW. Ontario	0.88 (1)	–	0.82 (1)	0.95	0.90
Illinois	0.54 (1)	0.74	–	0.72	0.78
Arkansas	0.70 (1)	0.94	0.81	–	0.85
Tennessee	0.76 (2)	0.90	0.76	0.86	–

their range. While the overall estimate of Φ_{ST} had marginal statistical significance and NCA indicated some phylogeographic structure, the majority of evidence indicated an essentially homogeneous population: Φ_{ST} was low (0.01); global estimates of θ and ρ were zero; none of the pairwise estimates of population differentiation were statistically significant; none of the indices of population differentiation were correlated with geographic distance between populations; and STRUCTURE provided strong support for a single genetic population. These results are similar to findings for other neotropical migrant songbirds, especially warblers. For example, molecular analyses revealed little if any population genetic structure in red-winged blackbirds (*Agelaius phoeniceus*, Ball et al. 1988) and common grackles (*Quiscalus quiscalus*, Zink et al. 1991). Several species of neotropical migrants show genetic differences between eastern and western or migratory and non-migratory populations, but little if any population genetic structure within either eastern or western North America (e.g. common yellowthroat *Geothlypis trichas*, Ball and Avise 1992; Lovette et al. 2004; song sparrow *Melospiza melodia*, Fry and Zink 1998; prairie warbler *Dendroica discolor*, Buerkle 1999; MacGillivray's warbler *Oporornis tolmiei*, Milá et al. 2000; Wilson's warbler *Wilsonia pusilla*, Kimura et al. 2002; Swainson's thrush, Ruegg and Smith 2002; yellow-breasted chat *Icteria virens* and Nashville warbler *Vermivora ruficapilla*, Lovette et al. 2004). However, a few species show more significant levels of genetic differentiation within eastern North America (e.g. yellow warbler, Gibbs et al. 2000; Milot et al. 2000; loggerhead shrike *Lanius ludovicianus*, Valliantatos et al. 2002; fox sparrow *Passerella iliaca*, Zink and Weckstein 2003).

Genetic homogeneity can result from recent expansion from a single refugial population (historical association) and/or from on-going gene flow. Several of our results indicate that cerulean warblers underwent a recent population expansion: the mismatch distributions, estimates of Tajima's *D*, nested clade analysis, and results from FLUCTUATE all indicate that the population has expanded (Tajima 1989a, b, Rogers and Harpending 1992; Rogers 1995; Kuhner et al. 1998; Templeton 2004). The mismatch distribution suggests that the expansion began ~13,000 years ago; although the mutation rate for Domain II for cerulean warblers is not know, rates as low as 1%/my still place the beginning of this expansion within the late Pleistocene (63,000 years ago). Analyses of microsatellite variation did not provide evidence for a recent change in population size, however, these analyses are less sensitive than mtDNA analyses: the effective population size of nuclear loci is four times higher than for mtDNA; and BOTTLENECK requires a large number of loci for a sensitive analysis (a minimum of 20 is recommended; Cornuet and Luikart 1996). Thus, although none of these tests are definitive on their own (e.g. negative values for Tajima's *D* may also result from selection; Tajima 1989a, b), together they suggest that the lack of population genetic structure in cerulean warblers is due at least in part to historical association. Lack of population genetic structure has also been attributed to post-Pleistocene population expansion in many other species of neotropical migrant birds (e.g. Ball et al. 1988; Zink et al. 1991; Milá et al. 2000; Ruegg and Smith 2002).

Results of the assignment tests suggest that the lack of population genetic structure in cerulean warblers may also be due at least in part to on-going gene flow. Six (4%) of 154 birds had high probabilities ($P > 0.99$) of being immigrants or the descendants of recent migrants. Although these (male) individuals could represent assignment errors, especially given the low level of differentiation among populations, assignment power with the present data exceeded 0.70 for most population pairs (Table 5), and tests of the general method indicate that accuracy is high even with weak population genetic structure and small numbers of loci (Rannala and Mountain 1997; Berry et al. 2004). Given that most birds sampled for the present study were males, and that dispersal in birds tends to be female-biased (Greenwood 1980; but see Gibbs 2000), contemporary dispersal may be even higher than suggested by these data.

Contemporary dispersal in cerulean warblers maybe explained by any or all of at least four hypotheses:

1. Dispersal rates may be naturally high. A lack of genetic structure has been attributed to high dispersal abilities in several other avian species (e.g. Haig and Oring 1988, Kvist et al. 1998).
2. Birds maybe moving in response to habitat loss and/or fragmentation (Wade and McCauley

1988; McCauley 1991; Porter 1999). For example, intensive agriculture has reduced forest cover in south-western Ontario from over 80% to only 11% since pre-settlement (OMNR 2000).

3. Cerulean warblers may have expanded their breeding range into eastern Ontario within the last century, either as invasion of new habitat or as re-occupation of the historical range as suitable habitat regenerates (McCracken 1992; Oliarnyk and Robertson 1996).
4. Some populations (e.g. eastern Ontario) may be serving as sources, providing recruits to sink populations elsewhere (see Introduction). The fact that five of six potential immigrants identified by IMMANC were sampled in Ontario appears to contradict this possibility; however, sample sizes and assignment power were highest for this population, biasing any potential conclusions based on the present data.

Rigorous evaluation of these hypotheses will require analysis of samples from additional sites throughout the breeding range, as well as larger sample sizes from each site and more loci.

Implications for conservation

Regardless of the reasons for the lack of population genetic structure in cerulean warblers, population decline and habitat fragmentation do not appear to have affected genetic variation in this species: none of the tests for genetic equilibrium detected a recent population decline, variation in mtDNA is similar to other bird species (reviewed in Baker and Marshall 1997), and heterozygosities are similar to levels for the same loci in yellow warblers (Dawson et al. 1997) and Swainson's thrushes (Gibbs et al. 1999). The estimate for the genetically effective population size generated by FLUCTUATE (2.4×10^6 females) is much higher than the current census (560,000 individuals); however, given rates of population decline and habitat loss in the last 100 years, this number is not unreasonable for pre-European settlement numbers (K.V. Rosenberg, Cornell Lab of Ornithology, pers. comm.). Thus, reduction of genetic variation does not yet appear to be a concern for this species.

Moritz (1994) defined genetic management units (MUs) as sets of populations that are demographically independent, differing significantly in allele frequencies at nuclear or mitochondrial loci, regardless of phylogenetic relationships among alleles. The absence of genetic structure among cerulean warblers from Ontario, Illinois, Arkansas and Tennessee and the evidence for contemporary gene flow among them suggest that these populations should be considered a single genetic management unit. However, samples from New England and the central United States need to be analyzed before management recommendations are made concerning birds in these populations. Furthermore, the question of whether contemporary dispersal is natural or is a response to anthropogenic disturbance should be addressed more rigorously. Although the existence of a source-sink system could not be assessed in this study, the high reproductive success of cerulean warblers in eastern Ontario (Oliarnyk and Robertson 1996; Jones et al. 2004), the variable demographics of this species (Oliarnyk and Robertson 1996; Jones et al. 2004), high dispersal ability, and apparently dynamic demographic history (present results) indicate that the potential exists for a source-sink population system, which should be explored further.

Acknowledgements

We are indebted to many people who assisted with sample collections or provided logistical support: J. Barg, C. Francis, J. Jones, C. Kellner, J. McCracken, W. McLeish, M. Mesures, S. Millard, C. Nicholson, M. Robbins, S. Robinson, J. Witherspoon, and C. Woodson. We also thank the staff of the Queen's University Biological Station, Meeman Shelby Forest State Park, Meeman Biological Station of the University of Memphis, Chickasaw National Wildlife Refuge, Anderson Tully Co., and the Mississippi Palisades State Park. Helpful discussions on laboratory techniques, analyses, and earlier drafts of this manuscript were provided by T. Birt, P. Boag, M. Damus, G. Ibarguchi, D. Jelinski, A. Patirana, A. Smith, T. Steeves, and M. Vallianatos. Financial support was provided by an NSERC postgraduate scholarship, James L. Baillie Student Research Award, American Ornithologists' Union Alexander Wetmore Research Award, and Queen's University Graduate Award to M.L.V, and NSERC Discovery Grants to V.L.F. and R.J.R.

Appendix 1

Frequencies of alleles at five microsatellite loci among five populations of cerulean warblers, and locus-specific estimates of allelic richness (R), observed heterozygosity (H_O), and estimated heterozygosity (H_E). Values in bold indicate a significant homozygote excess

Allele	EO	SO	IL	AR	TN
DPμ1					
136	0.08	0.13	0.13	0.06	0.12
140	0.01				
142		0.03			
144	0.05	0.03	0.04	0.04	0.02
146	0.01				
148	0.03		0.02	0.04	0.03
150	0.01	0.03	0.02		0.03
152	0.02	0.03	0.02	0.04	0.07
154	0.02	0.13	0.02	0.02	0.02
156	0.07	0.08	0.04	0.04	0.05
158	0.08	0.05	0.04	0.02	0.03
160	0.05	0.08	0.08	0.04	0.05
162	0.09	0.08	0.06	0.04	0.03
164	0.07	0.03	0.10	0.04	0.09
166	0.02	0.03	0.06	0.08	0.05
168	0.09	0.08	0.06	0.08	0.10
170	0.12	0.03	0.08	0.21	0.09
172	0.07	0.05	0.08	0.04	0.05
174	0.05	0.10	0.06	0.08	0.05
176	0.02	0.03	0.04	0.04	0.05
178	0.02				0.05
180	0.02		0.06	0.02	
182				0.02	
184		0.05	0.02	0.02	
R	16.1	18.0	17.6	17.9	16.8
H_O	**0.91**	0.95	0.96	1.00	0.97
H_E	0.94	0.95	0.95	0.93	0.95
DPμ3					
130	0.05	0.13	0.02	0.06	0.12
135	0.79	0.73	0.83	0.75	0.67
137	0.11	0.13	0.15	0.15	0.09
142	0.02			0.02	
144	0.02				0.03
149	0.01	0.03			0.05
151	0.01			0.02	0.02
165					0.02
R	4.8	4.0	2.8	4.7	6.3
H_O	0.38	0.55	0.31	0.46	0.55
H_E	0.36	0.45	0.30	0.42	0.53
Dpμ15					
123	0.02		0.02		
125	0.02		0.02		
127	0.01			0.02	0.02
129	0.05		0.02	0.04	
131	0.04	0.03	0.04		
133	0.01		0.04	0.04	0.05

Appendix 1. (*continued*)

Allele	EO	SO	IL	AR	TN
135	0.04		0.02	0.04	0.03
137	0.08	0.10	0.02	0.04	0.09
139	0.01	0.05			0.07
141	0.03	0.05	0.06	0.13	
143	0.11	0.05	0.10	0.02	0.05
145	0.12	0.28	0.25	0.10	0.14
147	0.07	0.23	0.08	0.08	0.05
149	0.05	0.08	0.12	0	0.05
151	0.11	0.05	0.02	0.08	0.09
153	0.09	0.03	0.04	0.17	0.02
155	0.09		0.10	0.08	0.14
157	0.02		0.02	0.04	0.02
159	0.01	0.03		0.06	0.09
161	0.01	0.05	0.06		0.02
163	0.01				0.09
167	0.01				
169	0.01				
171				0.04	
R	15.9	12.0	15.2	14.5	14.5
H_O	**0.47**	**0.45**	**0.73**	**0.29**	**0.52**
H_E	0.94	0.89	0.91	0.94	0.94
Dpμ16					
149	0.01	0.03	0.02		
151		0.03			0.02
153	0.09	0.15	0.06	0.06	0.02
155	0.38	0.20	0.33	0.44	0.33
157	0.12	0.10	0.10	0.13	0.16
159	0.10	0.23	0.12	0.15	0.14
161	0.06	0.13	0.12	0.06	0.12
163	0.09	0.03	0.10	0.06	0.12
165	0.03		0.08	0.04	0.05
167	0.03	0.03	0.02	0.02	0.03
169	0.04	0.03	0.02	0.04	
171	0.04	0.08	0.02		
173	0.02		0.02		0.02
175			0.02		
R	10.1	11.0	11.6	8.8	9.0
H_O	0.84	0.9	0.81	0.79	0.97
H_E	0.82	0.87	0.86	0.79	0.83
Swth28					
132	0.09		0.06	0.02	0.07
134	0.28	0.35	0.27	0.23	0.29
136	0.16	0.18	0.21	0.19	0.21
137	0.01			0.04	
138	0.42	0.43	0.40	0.48	0.33
140	0.02	0.05	0.04		0.03
142	0.02		0.02	0.02	0.07
146				0.02	
R	5.5	4.0	5.7	6.5	5.9
H_o	0.64	0.65	0.62	0.54	0.65
H_E	0.72	0.70	0.75	0.70	0.78

Appendix 2

Results of nested clade analysis of control sequences of cerulean warblers. "D_c" and "D_n" are clade distances and nested clade distances, respectively; "*I-T*" = distances between internal versus tip haplotypes; clades (including haplotypes) in grey are tip clades (see Figure 2); "*S*" and "*L*" represent distances that are significantly small or large, respectively. An asterisk indicates a nesting clade for which the exact contingency test indicated a non-random distribution of haplotypes or nested clades ($P < 0.05$); for the total cladogram, $\chi^2 = 42.1$, $P < 0.05$

Haplotypes			1-Step Clades		
No.	D_c	D_n	No.	D_c	D_n
1	905	880	1-1*	849S	860^S
2	0	786			
4	0	793			
9	1010	820			
10	0	786			
12	772	746			
13	643	698^S			
14	190	928^L			
15	0	901			
18	0	765			
20	0	765			
21	0	765			
23	0	989			
25	0	989			
27	0	989			
I-T	494^L	64			
3	1250	886	1-2	1040	750
5	0	542			
11	0	542			
19	0	1070			
I-T	1250	168			
6	1120	1010	1-3	1030	1020^L
22	0	600			
24	0	600			
I-T	1120	406			
7	920	836	1-4	863	717
8	0	657			
I-T	920	179			
16	-	-	1-5	0	1100
17	-	-			
26	-	-	1-6	0	1200
			I-T	-52	-42^S

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