Section 6 (Texas Traditional) Report Review

Form emailed to FWS S6 coordinator (mm/dd/yyyy): 3/13/2012

TPWD signature date on report: 3/12/2012

Project Title:
Resolving the conservation genetics of the black-capped vireo (Vireo atricapilla) with nuclear gene sequences.

Final or Interim Report? Final

Grant #: TX E-117-R-1

Reviewer Station: Arlington ESFO

Lead station concurs with the following comments: NA (reviewer from lead station)

Interim Report (check one):

- ☐ Acceptable (no comments)
- ☐ Needs revision prior to final report (see comments below)
- ☐ Incomplete (see comments below)

Final Report (check one):

- ☒ Acceptable (no comments)
- ☐ Needs revision (see comments below)
- ☐ Incomplete (see comments below)

Comments:

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-117-R-1

Endangered and Threatened Species Conservation

Resolving the conservation genetics of the black-capped vireo (*Vireo atricapilla*) with nuclear gene sequences

Prepared by:

Dr. Robert M. Zink

TEXAS PARKS & WILDLIFE

Carter Smith
Executive Director

Clayton Wolf
Director, Wildlife

12 March 2012

FINAL REPORT

STATE: ____Texas_____ GRANT NUMBER: ___TX E-117-R-1__

GRANT TITLE: Resolving the conservation genetics of the black-capped vireo (*Vireo atricapilla*) with nuclear gene sequences.

REPORTING PERIOD: ____1 Sep 09 to 28 Feb 12_

OBJECTIVE(S): To resolve the genetic status of Black-capped Vireo populations and whether they should effectively be managed as a single population or as multiple subpopulations.

Fiscal Year 1.

Task 1. DNA samples already in hand. The first month will involve readying the DNA samples and re-extracting if necessary.

Task 2. We will rank the loci by which are most variable, and choose the top 10 for use with all individuals.

Task 3. Sequencing nuclear loci for 40 individuals.

Fiscal Year 2.

Task 4. Sequencing of the nuclear loci for 66 individuals.

Task 5. Editing and aligning sequences, and cloning approximately 20 individuals for each gene to determine the extent of recombination.

Task 6. Conducting phylogenetic analyses on sequences from each locus.

Task 7. Prepare report from perspective of conservation implications of geographic patterns of variation in nuclear and mtDNA sequences with specific recommendations for managing Black-capped Vireo populatons from the standpoint of genetic differentiation (among populations) and genetic variation (within populations).

Significant Deviations:

None.

Summary Of Progress:

Please see Attachment A.

Location: Lab work at University of Minnesota, St. Paul, MN.

Cost: ___Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.__

Prepared by: _Craig Farquhar_____ Date: _12 March 2012

Approved by: _____ Date:_____12 March 2012__
C. Craig Farquhar

ATTACHMENT A

Final Report TX E-117-R-1

Resolving the Conservation Genetics of the Black-capped Vireo

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INTRODUCTION

The Black-capped Vireo (*Vireo atricapilla*) is a monotypic (i.e., without named taxonomic subspecies) endangered songbird with a limited distribution in Oklahoma, Texas, and Mexico. To guide management efforts, molecular genetic studies have been done to determine whether vireos in any part of the range constitute a distinct population segment that would require partitioning management effort among parts of the range. In addition, some studies attempted to deduce the nature of gene flow among the currently fragmented populations of the species, which could have implications for management of population connectivity.

Brief review of prior molecular genetic studies-- An alloyzme study (Fazio et al. 2004) reported that genetic differences existed among some populations, but Zink et al. (2010) believed these putative differences were probably artifacts. A major study of variation in microsatellite allele frequencies (Barr et al. 2008) found a very low but statistically significant level of genetic differentiation (Fst < 5%), and observed no partitioning of genetic variation into geographic regions (in opposition to the Fazio et al. 2004 result). However, Barr et al. (2008) plotted *F*st/(1 – *F*st) versus the log of geographic distance and claimed that although there was evidence of high levels of gene exchange among geographically proximate locations, that gene flow was reduced over larger areas. They interpreted this result to mean that preservation efforts should consider this information in managing remaining populations or restoring habitat.

Zink et al. (2010) presented the results of a mtDNA study that included Mexican breeding populations (these were lacking in Barr et al. 2008). They too found a very small Fst value, which they interpreted as indicating a lack of population structure. However, they determined there was an isolation-by-distance effect. In a later analysis (Zink et al. 2011), they plotted mtDNA *F*st/(1 – *F*st) versus the log of geographic distance and found a similar pattern to Barr et al. (2010) but concluded that it was not sufficiently robust upon which to base management recommendations. In fact, Zink et al. (2011:128) commented "Given that both analyses indicate some type of isolation-by-distance, it might be prudent to maintain viable populations within 100 km of each

other", which the latter qualified by stating (p. 128), "Our suggestion above to keep viable populations within 100 km of each other is precautionary, not a strong inference from either data set."

An exchange between the authors of the microsatellite study (Barr et al. 2008) and the mtDNA study (Zink et al. 2010) occurred in 2011 (Barr et al. 2011, Zink et al. 2011). Both sides maintained the veracity of their opinions.

RATIONALE AND RESULTS OF THE PRESENT STUDY

The present study was prompted by an ongoing debate in the literature over the use of molecular markers in conservation, and has direct implications for the Black-capped Vireo (Zink et al. 2010, 2011; Barr et al. 2008, 2011). One school of thought maintains that mtDNA provides the best chances of detecting recently isolated populations (Zink and Barrowclough 2008; Zink et al. 2010, 2011), which in essence are the currency of biodiversity. In short, mtDNA has a relative short coalescence time relative to nuclear markers, which means that mtDNA has a greatly enhanced probability of detecting recently evolved groups (Zink and Barrowclough 2008). Others (e.g., Barr et al. 2011, Edwards et al. 2005, Galtier et al. 2009) believe that mtDNA is biased by natural selection and can be misleading as an indicator of recent evolutionary history of populations. An important concern is that mtDNA does represent a single gene tree, albeit of the correct coalescence time, whereas reliance on any single locus (mtDNA or nuclear) could in theory produce a misleading estimate of species history. Thus authors have criticized, on the basis of the 'single locus argument,' analyses based solely on mtDNA. In fact, Galtier et al. (2009:4546) remarked that "mtDNA is perhaps intrinsically the worst population genetic and phylogenetic molecular marker we can think of." Empirical studies (e.g., McKay and Zink 2009), however, have shown that this sentiment is vastly overstated to the point of being ludicrous.

To address the concerns over basing conclusions on single loci (e.g., mtDNA) many authors have turned to analysis of microsatellites (e.g., Barr et al. 2008). Indeed one can assay many independent loci (unlike the case with mtDNA) and arrive at a consensus of multi-locus patterns of variation. However, these loci are nuclear in their inheritance and hence have a long coalescence period relative to mtDNA, and it is not clear that many loci with the incorrect coalescence times are indeed an advance over mtDNA. Therefore, despite having many loci, microsatellite analyses will not detect recently isolated groups more quickly than those detected by mtDNA. Nonetheless, hundreds of microsatellite studies have been published, many without comparative evidence from mtDNA. This means that these studies have potentially missed detection of recently isolated yet genetically distinct populations, the terminal units of biodiversity, because they lacked mtDNA data.

Apart from the long coalescence times, microsatellites have a number of other problems (reviewed in Brito and Edwards 2008, Zink 2010) that reduce their usefulness in phylogeography and conservation genetics. One of the main issues is that their alleles consist of stretches of highly mutable DNA that contain different numbers of short tandem repeat units rather than substitutions in a DNA sequence. Therefore, unlike mtDNA sequences, microsatellites cannot be analyzed using a powerful set of coalescence methods designed for sequence data that most agree are most appropriate for these fields.

The solution in part is to sequence nuclear loci, rather than use microsatellite allele frequencies. Although the loci still have long coalescence times relative to mtDNA, the data are DNA sequences that can be analyzed using coalescence methods. This is the approach we took here. It is the approach advocated by most recent authors in this field (Lee and Edwards 2008).

We sequenced nine nuclear loci for 124 individuals from 12 localities (Table 1; Fig. 1). The DNA samples we used for mtDNA had degraded considerably, resulting in the redesign of primers that allowed us to obtain relatively short stretches of sequence, and somewhat smaller sample sizes per locality (which are nonetheless perfectly adequate). K. Barr kindly supplied DNA samples from areas where our DNA samples were inadequate. We assumed there was no recombination in these short sequences. Each of the loci was variable, exhibiting from 5 to 55 alleles. Haplotype diversity (Hd) varied considerably, from a high of 0.96 (AETC locus) to 0.3 (Adams6). Nucleotide diversity (Pi) per locus followed a similar pattern. Geographic differentiation was measured by Fst, which ranges from 0 to 1 (in theory; see Barr et al. 2011), with low values indicating lack of population structure. Only 3 of the nine Fst-values were statistically different from zero; the overall average of 0.026 is not statistically significant. Importantly, this value is extremely similar to that obtained by analysis of microsatellites (0.021; Barr et al. 2008) and mtDNA (0.024; Zink et al. 2010).

Table 2 summarizes the levels of variation across populations. In general, there is little geographic variation in levels of genetic variability. For example, nucleotide diversity (pi), which takes into account differences in sample size, shows considerable uniformity. Thus, although the San Antonio sample (from Barr et al. 2008) is more variable than the others, there is no part of the range that appears genetically depauperate.

Geographic variation across loci. -- We used the program STRUCTURE (Prichard et al. 2000) to determine the number of groups based on the nuclear DNA sequences. In short, this program considers variation among loci simultaneously and attempts to determine whether there is more than 1 genetically distinct group represented in the data (Barr et al. 2008 used this for microsatellites and concluded that there was one group). The 9 nuclear introns were formatted as single-nucleotide polymorphism (SNP; see Manthey et al.

2011). The STRUCTURE analyses assumed an admixture model, correlated allele frequencies, and a fixed lambda value (which was inferred by setting $K = 1$ and allowing lambda to be estimated in an initial analysis). We analyzed the data for $K = 1$ to 4 with five replicates for each value of K. Each run contained 100,000 steps as burn-in followed by 500,000 steps. The ΔK was calculated *ad hoc* (Evanno et al. 2005) and used to identify the best estimate of K.

The ln-likelihoods (Table 3) for different values of K showed that the best explanation of the sequenced data occurred when K = 1, meaning that there is no differentiation among localities. For visualization, plots of K = 2 and K = 3 are shown (Fig. 2), which reinforce this interpretation: the Black-capped Vireo is a single evolutionary entity.

The lack of clear geographic structure does not automatically mean that the genetic information has no value in directing management. Barr et al. (2010, 2011) argued that their plot of genetic vs. geographic distance had significance for localities located ca. 100 km apart. We used the multi-locus sequence data to investigate the same issue. In Figure 3, we show the same plot as Barr et al. (2008) based on microsatellites and Zink et al. (2011) based on mtDNA. It is clear that there is no significant pattern.

Conclusions.--There are now multiple data sets for the Black-capped Vireo. Apart from the allozymes (Fazio et al. 2004), the microsatellites, mtDNA and nuclear DNA sequences all indicate that the species is a single evolutionary entity. The PI (Dr. Zink) is of the opinion that this new data set is consistent with their earlier statement (Zink et al. 2010: 802): *"we suggest that population management can operate free of taxonomic and genetic restrictions, focusing instead on demography and population viability to maintain large populations of Black-capped Vireos in several locations (as a buffer against disease, fire, changing climate conditions, and other factors). Existing populations should be prioritized for conservation value on the basis of demographic parameters, including population size and breeding success (factoring in effects of cowbird parasitism). Land-conservancy efforts should emphasize purchasing properties that connect high-value existing sites, even if these connecting properties will require active management to create appropriate habitat. Sites with smaller populations of Black-capped Vireos that are heavily parasitized by cowbirds or that function as sink populations are of lower priority in a world with limited conservation resources."*

Acknowledgments.--H. Vazquez-Miranda, M. Westberg, D. Blini, K. Barr, C. Farquhar, and A. Jones provided helpful assistance with various aspects of this project.

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Table 1. Summary statistics per locus. #bp = base pairs sequenced per locus, Hd = haplotype (or allele) diversity, Pi = nucleotide diversity, and Fst = degree of geographic differentiation (* = $P < 0.05$).

Locus	# bp	# alleles	Hd	Pi	Fst
Adams6	291	5	0.3	0.001	0.025
AETC	278	55	0.96	0.012	0.029*
Fibro	242	12	0.67	0.005	0.04
Gapdh	206	12	0.48	0.003	0.024
IQGap	250	16	0.81	0.006	0.045
lama2	275	7	0.51	0.002	0.022
MC1R	293	13	0.72	0.005	0.054*
TGFB2	236	15	0.82	0.006	0.038*
Trop6	277	14	0.65	0.003	0.002

Table 2. Summary statistics per locality (see Zink et al. (2010) and Barr et al. (2008) for locality information, and legend to Table 1 for abbreviations).

Locality	Ave number of alleles surveyed	pi	Hd	# alleles
OK	19.78	0.0043	0.68	5.2
BC	6.67	0.0043	0.69	3.3
CB	6.89	0.0044	0.57	3.1
DM	4.67	0.0032	0.44	2.1
DR	8.89	0.0042	0.68	3.6
FH	21.25	0.0039	0.52	5.1
IC	20.22	0.0050	0.68	5.8
KC	18.00	0.0046	0.59	5.1
KWMA	32.00	0.0051	0.68	7.6
QR	19.56	0.0036	0.59	4.8
SA	17.11	0.0064	0.71	5.9
MX	12.67	0.0054	0.74	4.5

Table 3. Values of K for different assumed numbers of populations of Black-capped Vireos. The highest LnL is for K=1. Twelve populations were tested so as to equal the number of sampling localities.

K	LnL
1	-2640.2
2	-2733.9
3	-2689.8
4	-2804.1
5	-2792.1
6	-2796.7
7	-2746.2
8	-2963.1
9	-2951.4
10	-2887.1
11	-2818.4
12	-2924.5

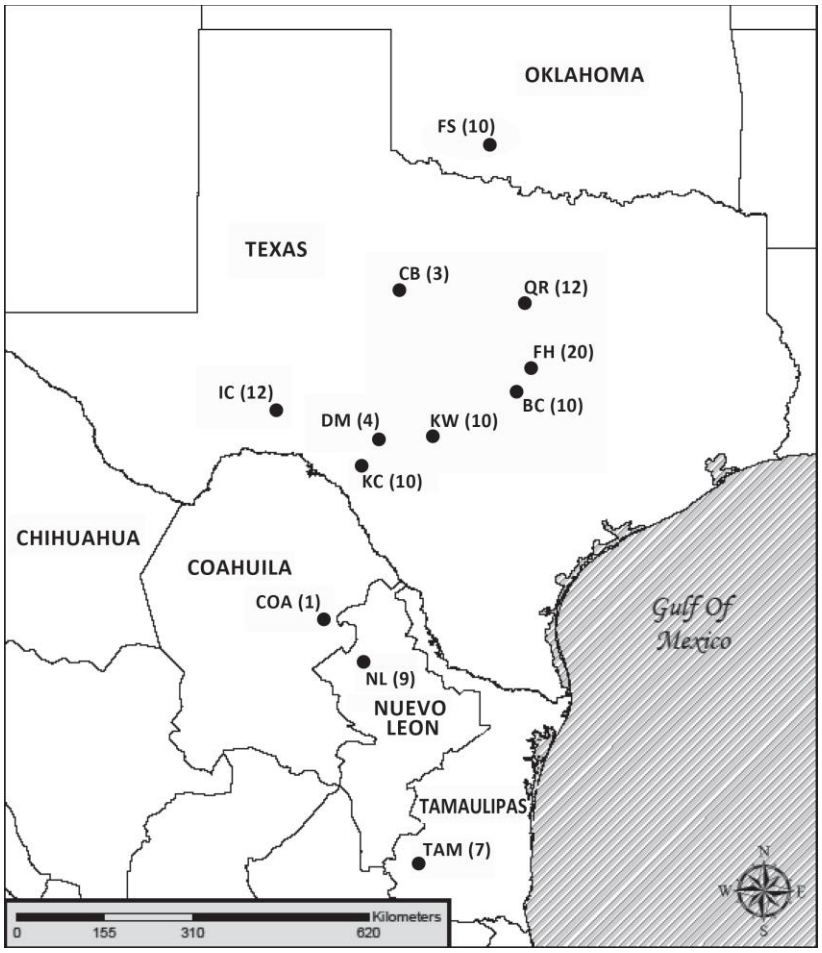
1) 

Fig. 1. Map of localities from Zink et al. (2010). For this analysis we added a sample from San Antonio (see Barr et al. 2010).

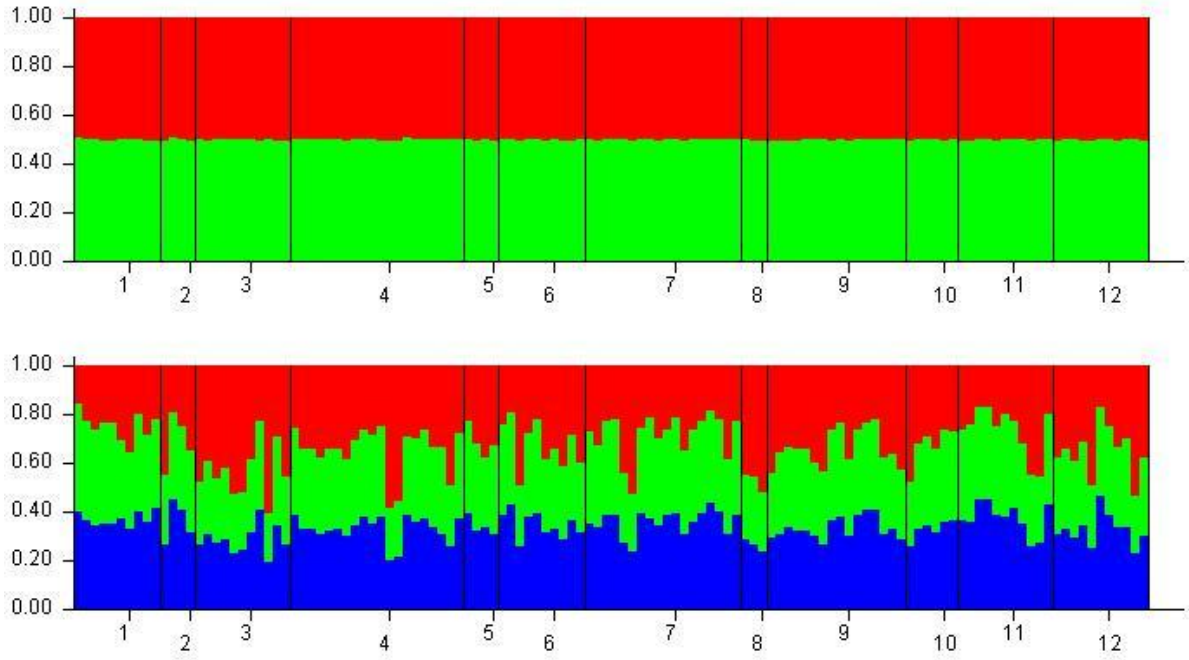


Fig. 2. Box plots showing distribution of genetic variation across nine nuclear loci and the 12 vireo localities. Top panel (K=2) and lower panel (K=3) show the lack of geographic structure to the genetic variation.

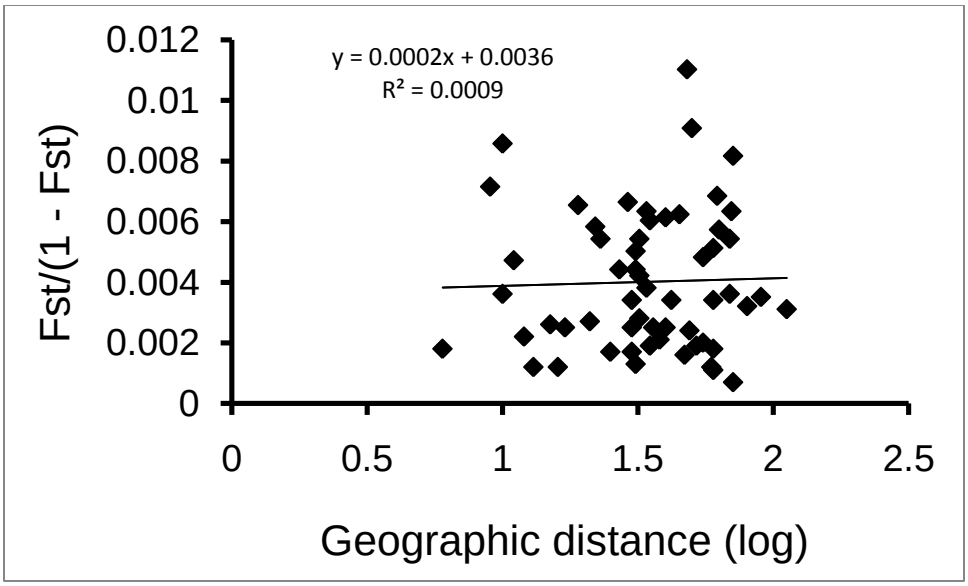


Fig. 3. Plot of genetic vs. geographic distance (based on sequences from nine nuclear loci) showing no significant isolation by distance.