Contract Number _____
CFDA Number 15.615

MEMORANDUM OF AGREEMENT
Between
TEXAS PARKS & WILDLIFE DEPARTMENT
And
The Regents of the UNIVERSITY OF MINNESOTA

This Memorandum of Agreement is made and entered into by and between Texas Parks and Wildlife Department, hereinafter referred to as TPWD, and the Regents of the University of Minnesota, a non-profit, educational institution, under the authority granted by Texas Parks and Wildlife Code, Section 11.017(a)(1)(C).

I. CONTRACTING PARTIES:

The Receiving Agency: Texas Parks and Wildlife Department (TPWD)

The Performing Entity: The Regents of the University of Minnesota (UMinn)

II. STATEMENT OF SERVICES TO BE PERFORMED (WORK):

UMINN shall conduct a project entitled *Resolving the conservation genetics of the Black-capped Vireo (Vireo atricapilla) with nuclear gene sequences* as outlined in the attached proposal, Attachment A, attached hereto and incorporated herein for all purposes.

At least one (1) TPWD employee will serve as a project advisor and co-author of all publications resulting from this research. The project advisor shall 1) Serve on any graduate committee as an official or unofficial member, 2) Serve as a co-author on publications resulting from this project as appropriate, and 3) Monitor progress of the project.

Authorship shall be determined based on substantial, actual and direct intellectual contributions to the work by the researchers. All authors should participate in writing the proposed manuscript or publication by reviewing drafts and each providing prior written approval of the final version. In the event of a dispute regarding contributions for purposes of authorship, the parties agree to negotiate a final version in accordance with these authorship principles in good faith. The University reserves the right to independently publish its results, whether or not there is a joint publication with TPWD, and University shall shall not indicate endorsement of the data by TPWD in any University publication without prior written approval.

All publications arising from this research shall acknowledge TPWD and UMINN funds or such fund as may supersede it in funding this project.

All data and analyses resulting from this project, and all information regarding the project, becomes the property of TPWD, and will be presented to TPWD in an electronic format determined by TPWD upon request, and not later than 28 February 2011. UMINN reserves the right to retain copies of all such electronic presentations and all data and analyses and to use, copy, publish, and modify the same, and to allow others to do so, for UMINN purposes. TPWD agrees to refrain from publishing any results or analysis of this study for two (2) years after the termination date of contract, after which TPWD may publish with no restriction.

With respect to such Intellectual Property as is (i) incorporated in the Work, or (ii) produced by UMINN or UMINN employees, subcontractors, or subcontractor's employees during the course of performing

the Work, UMINN hereby grants to TPWD a nonexclusive, perpetual, irrevocable, enterprise-wide license to use, copy, publish, and modify such Intellectual Property, and allow others to do so for TPWD purposes. UMINN shall secure all necessary intellectual property licenses from third parties and warrants that the Work and the intended use of the Work will not infringe any property rights of any third party.

Equipment and supplies purchased under this Contract shall be used, managed, and disposed of in accordance with the Texas Uniform Grant Management Standards (UGMS). Property records must be maintained on all capitalized or controlled property and equipment that include a description of the property, a serial number or other identification who holds title, the acquisition date, and cost of the property. These records must be provided to TPWD at time of acquisition. At least every two years and/or at project closeout a physical inventory of the property must be taken and the results reconciled with property records. TPWD will determine appropriate disposition of such property in accordance with UGMS.

Landowner Permission: In accordance with 12.103 of the Texas Parks and Wildlife Code, UMinn acknowledges that any work to be performed on private lands in Texas using these funds requires that grantees secure written permission from the private landowner(s) for the purposes of (i) access to the land, and (ii) use of data collected on that land. As such the Landowner Permission for Wildlife Research Form, is attached hereto as Attachment B, and is incorporated herein for all purposes.

Equipment and supplies purchased under this Agreement shall be used, managed, and disposed of in accordance with the Texas Uniform Grant Management Standards.

UMinn shall submit following guidelines provided by TPWD **(1) Interim Performance Report on or before 30 September 2010,** and **(2) a Final Report on or before 30 September 2011.** The Report shall then be forwarded to U. S. Fish and Wildlife Service (USFWS, Austin) for review and comments. TPWD will send revisions requested by USFWS, Austin, to the Principal Investigator for UMinn, who shall respond to TPWD in writing within sixty (60) days of receipt of revision request.

III. BASIS FOR CALCULATING REIMBURSABLE COSTS:

See Budget in Attachment A for details.

Allowable costs are restricted to those that comply with UGMS and additional state and federal rules and law. The Parties agree that all the requirements of the UGMS apply to this Contract, including the criteria for allowable costs. Additional federal requirements apply when federal funds are included in the reimbursement.

UMinn may make adjustments up to **ten percent (10%)** within cost categories without prior approval from TPWD, provided that the total reimbursable costs do not exceed total annual costs.

Expenditures for travel and travel-related expenses will be reimbursed at the official rate authorized by the State of Texas.

The cost of materials and supplies should be charged at their actual prices. Proper documentation is required for reimbursement.

Reimbursements are conditioned on the Contract activities being performed in compliance with the Contract.

IV. AGREEMENT AMOUNT:

The total amount of this Agreement shall not exceed: $ 65,117.00 (Sixty Five Thousand, one hundred and seventeen Dollars).

This contract is subject to cancellation, without penalty, either in whole or in part, if funds are not appropriated by the Texas Legislature, or otherwise made available, to the Texas Parks and Wildlife Department.

V. PAYMENT FOR SERVICES:

Receiving Agency shall pay for services received from appropriation items or accounts of the Receiving Agency from which like expenditures would normally be paid, based upon vouchers drawn by the Receiving Agency payable to Performing Entity.

For payment purposes, the Performing Entity (entity receiving payment) shall submit to the Receiving Entity (agency making payment) an invoice with the Performing Entity's RTI (for funds transfer at the Treasury) or a Purchase Voucher (for deposit in local bank account). The Receiving Agency will enter payment information into USAS.

Payments received by the Performing Agency shall be credited to its current appropriation item(s) or account(s) from which the expenditures of that character were originally made.

Any excess costs over the TPWD contribution toward one awarded contract cannot be submitted for reimbursement against another contract.

$13,023.40, which is twenty percent (20 %) of Federal Share funds, will be held until receipt and acceptance of annual/final report by TPWD.

VI. INVOICING:

Payments for service performed shall be billed: **Monthly**

SEND VOUCHERS TO:
Dr. Craig Farquhar
Wildlife Division
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, TX 78744

Invoices shall include details of work completed during the invoiced period. Backup documentation must be submitted for both the contract expenses incurred and the match amounts. Cost share must be documented on each invoice. A sample Invoice with match documentation is attached hereto as Attachment C.

If the invoice or backup documentation is not complete it will delay the processing of your invoice.

The entire match amount must be obligated by the end of the period of performance or the performing agency risks a proportionate reduction in the amount of federal funds awarded under this Contract. If Performing Agency does not meet the match requirement of this agreement, the amount of federal funds to be disbursed will be proportionately lowered or, if already disbursed, a refund of federal funds will be required.

All invoices and reports must be received within 60 days of end of each fiscal year or termination of contract. Invoices not received within this time frame may not be paid.

VII. CONTACT INFORMATION

TPWD PI Name & Contact Info
Craig Farquhar
Endangered Species Grants Coordinator
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, Texas 78744
512-389-4933 office
512-389-8043 fax
craig.farquhar@tpwd.state.tx.us

TPWD Contract Point of Contact
Tammy Dunham, CTPM, CTCM
Contract Specialist
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, Texas 78744
512-389-4752 office
512-389-4677 fax
tammy.dunham@tpwd.state.tx.us

UMinn PI Name & Contact Info	UMinn Contract Point of Contact
Robert M. Zink, Ph. D.	April Coon
100 Ecology Bldg	200 Oak St. SE, Suite 450
University of Minnesota	Regents of the University of Minnesota
St Paul, MN 55108	Minneapolis, MN 55455
612 624 7207 Office	612-624-7021
612 624 6777 Fax	612-624-4843 (fax)
zinkx003@umn.edu	John5885@umn.edu

VII. TERM OF AGREEMENT:

This Agreement is to begin Upon Signature by Both Parties, and

shall terminate February 28, 2012

An extension to this Agreement may be granted with prior written approval by TPWD

VIII. MISCELLANEOUS PROVISIONS:

Definitions: As used throughout this contract, the following terms shall have the meaning set forth below:

A. Capitalized Property is real or personal property that have an estimated life of greater than one year. A capital asst has a value equal to or greater than the capitalization threshold established for that asset type.

B. Controlled Property is a capital asset that has a value less than the capitalization threshold established for that asset type, however due to its high-risk threshold, is required to be reported to SPA. The Comptrollers controlled assets list can be found online at: https://fmx.cpa.state.tx.us/fmx/spa/classcosed/control.php.

C. Contractor shall mean that firm, provider, organization, individual or other entity performing service(s) under this contract, and shall include all employees of the Performing Entity.

Termination: This Agreement is subject to cancellation, without penalty, either in whole or in part, if

1. Funds are not appropriated by the Texas Legislature, or otherwise made available, to TPWD;
2. Performing Entity fails to comply with the terms and conditions of this Agreement; or
3. Performing Entity fails to comply with the provisions of applicable state or federal laws or regulations.

Audit: Performing Entity understands that acceptance of funds under this Agreement acts as acceptance of the authority of the State Auditor's Office, TPWD or any successor agency, to conduct an audit or investigation in connection with those funds. Performing Entity further agrees to cooperate fully with the above parties in the conduct of the audit or investigation, including providing access to any information the state auditor considers relevant to the investigation or audit. Performing Entity shall ensure that this clause concerning the authority to audit funds received indirectly by subcontractors through the Performing Entity and the requirement to cooperate is included in any subcontract it awards. In Agreements involving federal funds, the right to audit provision of the Agreement includes the right for the applicable federal agencies and the federal Office of Inspector General to audit.

Dispute Resolution: In accordance with Chapter 2261 of the Texas Government Code, the following Schedule of Remedies applies to this Agreement in the event of substandard performance or other failure to conform to the requirements of the Agreement or applicable law.

(a) Reject the substandard performance and request corrections without charge to TPWD.
(b) Issue a notice of substandard performance or other non-conforming act or omission.
(c) Request and receive the return of any over payments or inappropriate payments.
(d) Reject associated reimbursement requests and suspend payments, pending accepted revision of substandard performance or non-conformity. Note: Funds may be retained by TPWD for

recovery of administrative costs or returned to funding source as authorized by agreements with the funding source and by state or federal law.

(e) Suspend all or part of the Agreement, pending accepted revision of substandard performance or non-conformity.

(f) Terminate the Agreement, and demand and receive return of all equipment purchased of contract funds, return of all unexpended funds, and repayment of expended funds.

TPWD may avail itself of any remedy or sanction provided in this Agreement or in law to recover any losses arising from or caused by Performing Entity's substandard performance or any non-conformity with the Agreement or the law. Prior to initiating suit in relation to this Agreement, however, the parties shall attempt informal resolution, with elevation to the level of Agency Head and Vice President for Research, respectively; and, if informal resolution is unsuccessful, will engage in good faith mediation with an agreed third party neutral.

Performing Entity shall carry on the Agreement Activities and adhere to the progress schedule during all disputes or disagreements with TPWD unless ordered to stop the Agreement Activities. No Agreement Activities shall be delayed or postponed pending resolution of any disputes or disagreements.

Neither payment by TPWD nor any other act or omission other than an explicit written release constitutes a release of Performing Entity from liability for losses under this Agreement.

Other Law: Performing Entity shall give all notices and comply with all laws and regulations applicable to the Agreement and the grant of federal funds, including but not limited to 43 CFR 12, 2 CFR 215, 2 CFR 230, 40 CFR 34 (New Restrictions on Lobbying), OMB Circular A-133 and A-122, E.O.s 12549 and 12689, the National Environmental Policy Act, and Section 7 of the Endangered Species Act. As part of this Agreement, Performing Entity further agrees to comply with state and federal assurances attached hereto as Attachments D and E, respectively, and incorporated herein for all purposes.

Assignment: The Performing Entity shall not assign or subcontract the whole or any part of the Agreement without TPWD' prior written consent.

Entire Agreement; Modifications: The Agreement supersedes all prior agreements, written or oral, between Performing Entity and TPWD and will constitute the entire Agreement and understanding between the parties with respect to the subject matter hereof. The Agreement and each of its provisions will be binding upon the parties and may not be waived, modified, amended or altered except by a writing signed by TPWD and Performing Entity.

Venue and Governing Law:

This Agreement, and any disputes arising under it, shall be governed by and construed in accordance with the laws of competent jurisdiction, which shall be the forum for any legal actions arising from or incident to this Agreement. Pending resolution of any dispute, Subcontractor shall proceed diligently with the performance of its obligations under this Agreement.

University of Minnesota agrees to comply with all relevant state and federal statues and regulations, if any, in performing its obligations under this Agreement.

RECEIVING AGENCY	PERFORMING ENTITY
TEXAS PARKS AND WILDLIFE DEPARTMENT	Regents of the UNIVERSITY OF MINNESOTA
By: Michele Martinets, CTPM	By: David Hagen
Authorized Signature	Authorized Signature
Manager	David Hagen Associate Director
Title	Title
Date: 1/6/10	Date: 12/29/09

ATTACHMENT A

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

Principal Investigator: Dr. Robert M. Zink, University of Minnesota, St. Paul, MN

Need: Genetic data should clarify and not impede conservation planning. Unfortunately, in the case of the Black-capped Vireo (*Vireo atricapilla*), this has not been the case. Conflicting genetic data sets, or at least interpretations of them, have left an unclear direction for planning for the species continued survival. The objective of this proposal is to provide a remedy through a nuclear gene sequencing study.

The Black-capped Vireo is a small passerine bird that breeds in thornscub and shrubland habitats in Oklahoma and Texas in the US and in Coahuila, Nuevo Leon, and Tamaulipas in Mexico. This species formerly occurred in south-central Kansas until the early 1900s. Black-capped Vireo populations declined due primarily to habitat loss and cowbird parasitism (Grzybowski 1995), and have subsequently increased (Wilkins et al. 2006). The species, however, is still of conservation concern, currently considered Endangered within Mexico and the United States, Vulnerable by the IUCN Red List, and G2G3 by NatureServe.

Three kinds of genetic data have been gathered on Black-capped Vireo: allozymes, microsatellites and mtDNA sequences. An allozyme analysis (Fazio et al. 2004) of three Texas populations and one Oklahoma population found significant population differentiation, contrary to nearly all of the hundreds of allozyme papers on avian populations. Apparently the allozyme data included two loci that were under selection, biasing their result of population differentiation. Barr et al. (2008) reported on 15 microsatellite loci scored from birds sampled in Oklahoma and Texas; they lacked samples from Mexico. They suggested that their data showed "high interpopulation differentiation compared to other migratory birds" and that "Population differentiation is prevalent throughout the range of the black-capped vireo." They concluded that their "finding of locally limited gene flow implies they [black-capped vireo populations] might be treated as a metapopulation for management purposes." An mtDNA analysis of 106 individuals from 15 populations from Oklahoma, Texas, and Mexico (Jones et al. submitted) discovered a low level of genetic differentiation, and they concluded "Given the lack of subdivision in morphological, microsatellite, and mitochondrial data we suggest that maintaining large populations in several locations would effectively conserve genetic diversity." Therefore, conservation recommendations based on the two most common data sets used in conservation genetics, mtDNA and microsatellites, appear contradictory (see also Appendix below).

Although microsatellites are being used commonly in conservation, there are important limitations with their use in this field that are often not discussed. Alleles might not be identical by descent (a pre-requisite for their use) owing to different gain/loss events leading to the same allele size. The allele frequencies cannot be used to make a phylogenetic tree, and even if individuals are pooled (which makes an implicit assumption about their common ancestry) the resulting phenogram cannot be rooted. Recognizing that mtDNA sequences yield a single, albeit highly informative phylogenetic gene tree, many researchers are turning to sequence-based analyses of nuclear loci (Carstens and Knowles 2007, Liu et al. 2008). These provide independent gene trees that can be analyzed in the same way as mtDNA sequences, yielding truly complementary data sets for inferences about conservation. Although nuclear loci tend to exhibit low variability, by sequencing enough loci we can effectively test mtDNA results.

I propose to use existing samples of DNA that were used for our previous mtDNA analysis (Section 6 grant E-57) to sequence 10 nuclear gene loci for Black-capped Vireo populations from Oklahoma, Texas and Mexico. These sequences will be compared directly to mtDNA sequences. The null hypothesis is that there is no significant population differentiation, which will be tested with analysis of molecular variation (AMOVA) and phylogenetic gene trees. If the null hypothesis is not rejected, management can proceed without special consideration of genetic factors. The Black-capped Vireo will be one of the first species for which a comprehensive molecular data set -- consisting of sequences from mtDNA and the nuclear genome -- will be available. It is important that the conflict between previously surveyed molecular markers (mtDNA, microsatellites) be resolved, and sequencing nuclear genes will accomplish this because they yield information directly comparable to mtDNA.

Objective: 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.

Expected Results and Benefits: Given current information on the genetics of Black-capped Vireo populations, it is unclear how they should be managed. For example, if populations are clearly genetically isolated, so that they are reciprocally monophyletic on a gene tree, they could be managed as distinct population units. Conversely, if the history of populations is one lacking geographic isolation and consequently the populations are genetically undifferentiated, then management plans can proceed assuming populations are interchangeable. Although there might be minor ecological differences among populations that are not reflected in genetic studies, it is unlikely that they present important reasons for preservation. That is, it is likely that every geographically isolated population could be shown to have slight ecological differences. Plus, the concept of ecologically exchangeability is too vague to be implemented in practice (Zink 2007).

At the present time, microsatellite and mtDNA data are interpreted in conflicting ways. Barr et al. (2008) state that there are important genetic differences among populations based on microsatellite loci whereas Jones et al. (submitted) interpret the mtDNA data as indicating the populations are essentially genetically homogeneous. Part of the conflict stems from the different aspects of the two data sets. Microsatellite loci are chosen if they exhibit a high number of alleles, and with sufficiently large numbers of loci, populations, and individuals, slight differences in allelic frequencies can become statistically significant. This is the case with the Barr et al. (2008) study. Their overall Fst, the amount of genetic variance distributed among populations, is 2.1%. That is, 97.9% of the genetic variation has nothing to do with geographic structure. It is hard to make a case for a management plan to protect 2.1% of the variation, especially given congressional direction to use the ESA sparingly. However, it is nonetheless the case that their frequency differences are statistically different and Barr et al. (2008) interpret them as biologically significant, and relevant for management of vireo populations. The mtDNA results suggest that vireo populations can be managed without special concern for genetic distinctiveness or differing levels of genetic variability.

It is difficult to make an explicit comparison between microsatellites and mtDNA sequences. The reason is that microsatellite data consist of allelic frequencies with an unclear underlying evolutionary model, whereas as both nuclear and mtDNA gene sequences evolve under similar well understood rules. The nuclear gene sequences that will be gathered in this project will be compared directly to the mtDNA sequences. It will be straightforward to determine if the two sets of sequences are in conflict. If all gene trees suggest a lack of structure, and if the amount of variation distributed among localities is insignificant, or at least less than 10%, the data sets will be considered in agreement that there is no biologically significant population structure in the black-capped vireo. At present, neither mtDNA nor microsatellite data support any DPS designation within black-capped vireo, and this too is directly testable with nuclear gene sequences. If nuclear loci corroborate this assessment, it will mean that management plans for the vireo need not take genetics specifically into account.

Approach:

Task 1. DNA samples are already available from 106 individuals representing the breeding distribution. In the case that any DNA samples are depleted, we will re-extract feathers already in hand. The first month will involve readying the DNA samples and re-extracting if necessary. Fiscal Year 1.

Task 2. Our lab has approximately 40 sets of primers that amplify nuclear gene introns (noncoding). We will take 5 individuals each from OK, TX and Mexico and determine which primer sets provide the best amplification, and which are most variable. This will often involve trying several different amplification conditions. We will rank the loci by which are most variable, and choose the top 10 for use with all individuals. Fiscal Year 1.

Task 3. Sequencing nuclear loci for 40 individuals. This involves sufficient time for repeating experiments that did not work (about a 10% rate is standard). We strive to insure that all sequences are complete. Fiscal Year 1.

Task 4. Sequencing of the nuclear loci for 66 individuals. This involves sufficient time for repeating experiments that did not work (about a 10% rate is standard). We strive to insure that all sequences are complete. Fiscal Year 2.

Task 5. Editing and aligning sequences, and cloning approximately 20 individuals for each gene to determine the extent of recombination. Recombination can result in pruning lengths of some sequences. Fiscal Year 2.

Task 6. Conducting phylogenetic analyses on sequences from each locus. We will make separate gene trees for each locus. We will also use the software BEST (Liu et al. 2008; Bayesian estimate of species trees) that will analyze sequences in concert to estimate an overall tree. We will also estimate the degree of population subdivision using standard analyses such as AMOVA (Fst). Values will be compared with those from mtDNA. Note that the two valules are

not directly comparable, rather they are related by the following formula: $F_{st\text{-}nuc} = F_{st\text{-}mt}/(4 - 3F_{st\text{-}mt})$ Fiscal Year 2.

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). Fiscal Year 2.

APPENDIX. Further information on the importance of choosing the correct genetic analysis for conservation assessment

Genetic information has become a routine component of conservation decisions. The goal of most conservation genetics research is to determine whether populations are discrete and qualify as distinct population segments, or are more likely characterized as single entities owing to a history of gene flow and population interconnectedness. However, results may be intermediate and their interpretation is anything but routine. This is especially true in cases where large samples of individuals render small differences in allelic frequencies statistically significant (see above referenced discussion of Barr et al. 2008). Obviously the question is whether they are biologically significant. Little direct guidance exists on this topic in the conservation literature. One group suggests that significant differences are important no matter the magnitude of genetic differentiation. Alternatively, I suggest that a more conservative view is useful, one requiring a gene tree to show that populations are truly evolving independently.

A recent complication in conservation genetics arises from the comparison of different molecular markers. For two decades, mitochondrial DNA (mtDNA) has been used to determine if populations are genetically differentiated. For many reasons, mtDNA is an appropriate choice for this endeavor (Zink and Barrowclough 2008). Sequences of individual's mtDNA can be analyzed phylogenetically, and rooted with an appropriate outgroup. Importantly, mtDNA exists as a single strand (haploid) and it is inherited as one unit from the mother (matrilineal). In contrast, nuclear DNA exists as a double strand (diploid) and is inherited from both parents (biparental). Consequently, the number of generations backward in time it takes to discover a common ancestral sequence among populations (coalescence on a monophyletic gene tree) with mtDNA is one-fourth that required for nuclear DNA such that mutations leading to phylogenetic differences will be more rapidly known from mtDNA. In other words, for recently isolated populations, mtDNA gene trees will show they are evolving independently, whereas nuclear trees cannot, simply because of the difference in their inheritance (and time to coalescence). Thus, mtDNA is *the* choice for recently isolated groups. When the resolved mtDNA gene tree is superimposed over geography, patterns of isolation among populations can be readily visualized. MtDNA has therefore gained long-standing respect in detecting geographically segregated groups of threatened or endangered populations in nature. For example, mtDNA sequences clearly distinguish three independently evolving groups of Spotted Owls (*Strix occidentalis*; Barrowclough et al. 2005).

Recently, analyses of microsatellite loci (polymorphic simple sequence repeats in nuclear DNA) have been used to investigate problems in phylogeography, including endangered and threatened species. It is not clear, however, that these are better suited to the questions posed in conservation genetics. For example, to produce a hierarchical gene tree, the essence of phylogeography, one must group samples into *a priori* units, which make an implicit assumption that individuals in these units share a common ancestor. Trees produced from these loci cannot be rooted easily, if at all, resulting in trees that can be explicitly misleading (as in a study of Spotted Owls by Haig et al. (2001)). Importantly, because microsatellite loci are located in the nucleus, their coalescence time precludes capture of recent isolation events (see above). It is therefore unclear why so many studies have turned to analyses of microsatellite allele frequencies, when many of the important questions deal with recently isolated populations. Some authors state that because of the large number of alleles detected at some loci that microsatellites "evolve faster" than mtDNA sequences. This, however, is a misconception based on confusing the mutation rate with coalescence time. Having more alleles has nothing to do with coalescence time, or the ability of a gene tree to detect population subdivision. In a review, Zink and Barrowclough (2008) found that the most common result among numerous studies examined was a structured but relatively "shallow" mtDNA gene tree and a lack of differentiation in microsatellite loci, which they attributed to insufficient time in isolation for nuclear loci to become different between localities. That is, if isolation is recent, one *expects* only the mtDNA gene tree to show it. If a nuclear gene tree does not show structure, it is not in conflict with a mtDNA tree that shows shallow structure. It is simply a result of the difference in the inheritance of the two types of genetic markers.

It is my opinion that mtDNA gene trees showing either deeply structured geographically sensible groupings (e.g. spotted owls), or the opposite, no geographic structure (e.g. California Gnatcatcher), will prove correct upon testing with nuclear sequences. It is prudent, especially in important cases involving threatened and endangered species, to test mtDNA gene trees with nuclear sequencing – that is certainly the consensus in the literature of phylogeography and conservation genetics. By examining nuclear gene sequences (non-microsatellite) and comparing them to our previous mtDNA results,

this study will circumvent the problems in comparing two different, relatively incomparable, types of data, microsatellites and mtDNA. Appropriate, time-tested principles and methods of phylogeography will be applied in a consistent way to our nuclear and mitochondrial sequences. This ultimate comparative genetics approach, currently lacking in the field of conservation, will usher in an important era in making robust conservation decisions from independent genetic datasets that are directly comparable, and resolve the conflicting interpretations over microsatellite and mtDNA datasets in Black-capped Vireo.

Project Personnel:

Robert M. Zink, Ph. D.
100 Ecology Bldgj.
University of Minnesota
St Paul, MN 55108
612 624 7207
fax 612 624 6777
email: zinkx003@umn.edu

Literature Cited:

Barr, K.R., K. L. Lindsay, G. Athrey, R. F. Lance, T. J. Hayden, S. A. Tweddale, and P. L. Leberg. 2008. Population structure in an endangered songbird: maintence of genetic differentiation despite high vagility and significant population recovery. Mole. Ecology 17:3628-3639.

G. F. Barrowclough, J . G . Groth, L. A. Mertz and R . J. Gutiérrez. 2005. Genetic structure, introgression, and a narrow hybrid zone between northern and California spotted owls (*Strix occidentalis*). Molecular Ecology 14:1109–1120.

Carstens, B. C. and L. L. Knowles. 2007. Shifting distributions and speciation: species divergence during rapid climate change. Mole. Ecol. 16:619-627.

Fazio, V.W. III, D. B. Miles, and M. M. White. 2004. Genetic differentiation in the endangered black-capped vireo. Condor 106:377-385.

Grzybowski, J. A. 1995. Black-capped Vireo (*Vireo atricapilla*), *In* The Birds of North America, No. 181 (A. Poole and F. Gill, eds.). The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D. C.

Haig M, R.S. Wagner, E.D. Forsman, T.D .Mullins. 2001. Geographic variation and genetic structure in Spotted Owls. Conservation Genetics, **2**, 25-40.

Liu, L., D. K. Pearl, R. T. Brumfield, S. V. Edwards. 2008. estimating species trees using multiple-allele dna sequence data. Evolution 62:2080-2091.

Wilkins, N., R. A. Powell, A. A. T. Conkey, and A. G. Snelgrove. 2006. Population status and threat analysis for the black-capped vireo. US Fish and Wildlife Service, Region 2, Albuquerque, New Mexico.

Zink, R. M. 2007. Ecological exchangeability versus neutral molecular markers: the case of the great tit (*Parus major*). Animal Conservation 10:369–373.

Zink, R. M. & G. F. Barrowclough. 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology 17:2107–2121.

Zink, R. M., G. F. Barrowclough, J. L. Atwood, and R. C. Blackwell-Rago. 2000. Genetics, taxonomy and conservation of the threatened California Gnatcatcher. Conservation Biology 14: 1394–1405.

Estimated Cost:

Description	Federal	Match	Total
YEAR 1			
½ time salary for technician	16943		16943
Fringe Benefits for technician (32.7%)	5540		5540
Lab supplies (PCR, sequencing, primers - $11.50/bird/locus) X 10 loci X 40 individuals	4600		4600
Total direct costs:	27083		
Indirect costs (15%)	4063		4063
Indirect costs (51%-15%)		9750	9750
Zink salary (1%)		1090	1090
Zink fringe (30.4%)		331	331
Year 1 total	31,146	11,171	42,317
YEAR 2			
½ time Salary for technician	17536		17536
Fringe Benefits for technician (32.7%)	5734		5734
Lab supplies (PCR, sequencing - $9.50/bird/locus) X 10 loci X 66 individuals	6270		6270
Total direct costs	29540		29540
Indirect costs (15%)	4431		4431
Indirect costs (51%-15%)		10635	10635
Zink salary (1%)		1129	1129
Zink fringe (30.4%)		343	343
Year 2 total	33971	12107	46,078
Cumulative Years 1 and 2 Total	**65,117**	**23,278**	**88,395**

TEXAS PARKS & WILDLIFE

Attachment B

LANDOWNER PERMISSION FOR WILDLIFE RESEARCH

(Pursuant to Section 12.103 of the Texas Parks and Wildlife Code)

1. **Use of Information:** I hereby grant approval for Texas Parks and Wildlife Department employees to enter property I own or manage to conduct scientific investigations and research on wildlife and to record and use (such as in analyses) site-specific information from the property. This may include placing that information onto a topographic map and entering the information into a Department database. Thus, the information could be viewed by the public.

_____ _____
(Landowner or authorized agent) (Date)

2. **Reporting of Information:** I hereby grant approval for Texas Parks and Wildlife Department employees to report (such as in publications or technical reports) the above approved information in a manner that permits identification of the location of the specific parcel of property I own or manage.

_____ _____
(Landowner or authorized agent) (Date)

3. **Other Conditions**: List any other conditions that apply to this approval.

_____

_____

4. **Name and Address:**

_____
(Name of Landowner or Authorized Agent)

_____
(Address)

_____
(City, State, Zip)

5. **Optional:**

_____
(Name of Ranch or Tract)

_____ _____
(County) (Acreage)

_____ _____ _____
(Home Phone) (Office Phone) (FAX)

Texas Parks and Wildlife Department maintains the information collected through this form. With few exceptions, you are entitled to be informed about the information we collect. Under Sections 552.021 and 553.023 of the Texas Government Code, you are also entitled to receive and review the information. Under Section 559.004, you are also entitled to have this information corrected. For assistance call 512-389-4978.

Attachment C

Sample Invoice with Match (on vendor letterhead)

ADDRESS:
Send to the address of the contact

person on the contract.

Invoice Date: MM/DD/YY

Invoice #: #####

Payment amount for Performance Period: **$5,865.00**
(Total Costs in Column B, Expenditure Categories)

RE: Contract #_____
Performance Period: MM/DD/YY through MM/DD/YY
(same as Performance Period in Column B)

	(A)	(B)	(C)	(D)
Expenditure Categories	**Contract Budget**	**Performance Period for MM/DD/YY through MM/DD/YY**	**Cumulative Expenses**	**Balance of Budget as of MM/DD/YY**
Salaries	$10,000.00	$2,000.00	$2,000.00	$8,000.00
Fringe Benefits (25%) *	$2,500.00	$500.00	$500.00	$2,000.00
Professional and Contracted Services	$5,000.00	$2,000.00	$2,000.00	$3,000.00
Travel	$1,000.00	$200.00	$200.00	$800.00
Supplies	$2,500.00	$400.00	$400.00	$2,100.00
Equipment	$5,000.00	$0.00	$0.00	$5,000.00
Total Direct Costs	$26,000.00	$5,100.00	$5,100.00	$20,900.00
Indirect (if allowable) 15% *	$3,900.00	$765.00	$765.00	$3,135.00
Total Costs	$29,900.00	$5,865.00	$5,865.00	$24,035.00
Match Expenditures				
Salaries	$10,000.00	$2,000.00	$2,000.00	$8,000.00
Fringe Benefits	$2,500.00	$500.00	$500.00	$2,000.00
Professional and Contracted Services	$5,000.00	$2,000.00	$2,000.00	$3,000.00
Travel	$1,000.00	$200.00	$200.00	$800.00
Supplies	$2,500.00	$400.00	$400.00	$2,100.00
Equipment	$5,000.00	$0.00	$0.00	$5,000.00
Total Direct Costs	$26,000.00	$5,100.00	$5,100.00	$20,900.00
Indirect (if allowable)	$3,900.00	$765.00	$765.00	$3,135.00
Total Costs	$29,900.00	$5,865.00	$5,865.00	$24,035.00

* Fringe Rates & Indirect Rates must be approved in the grant application process with TPWD.

I, subrecipient/vendor, certify that this invoice is correct and that matching funds (if required in the contract) have been provided and will be subject to audit under OMB Circular A-133. I further certify that this invoice has not been previously paid.