

Population structure in an endangered songbird: maintenance of genetic differentiation despite high vagility and significant population recovery

KELLY R. BARR,* DENISE L. LINDSAY,† GIRI ATHREY,* RICHARD F. LANCE,†
TIMOTHY J. HAYDEN,‡ SCOTT A. TWEDDALE‡ and PAUL L. LEBERG*

*Department of Biology, University of Louisiana, Lafayette, LA 70504, USA, †Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, MS 39180, USA, ‡Construction Engineering Research Laboratory, US Army Engineer Research and Development Center, Champaign, IL 61826, USA

Abstract

Black-capped vireos (*Vireo atricapilla*), an endangered, migratory species dependent upon early successional habitat, have experienced significant recovery since its protection. In light of its vagility and known increase in population size and range, limited genetic differentiation would be expected in the species. Using 15 microsatellite loci and an extensive sampling regime, we detected significant overall genetic differentiation ($F_{ST} = 0.021$) and high interpopulation differentiation compared to other migratory birds. Although proximate sites (separated by < 20 km) tended to be genetically similar, there was no apparent association of either geographical distance or landscape attributes with differentiation between sites. Evidence of a population bottleneck was also detected in a site located near other large concentrations of birds. Although black-capped vireos are capable of large-scale movements and the population has experienced a recent expansion, dispersal appears too insufficient to eliminate the genetic differentiation resulting from restricted colonization of ephemeral habitats.

Keywords: black-capped vireos, endangered species, gene flow, microsatellites, recovering populations

Received 1 April 2008; revision received 12 June 2008; accepted 18 June 2008

Introduction

Across the globe, many species dependent upon disturbance and habitat succession are in decline (Hunter *et al.* 2002; Neill 2007; Warren & Buttner 2007). Besides the direct conversion of natural vegetation communities for other land uses, natural disturbance regimes, such as fire, have been disrupted by human activities. When disturbance is limited, vegetative succession continues serially into later stages, eliminating early successional habitats, such as grasslands and shrub lands.

Because early successional habitats are inherently transitory, the ability of species dependent upon those habitats to colonize new sites is critical for the long-term persistence of that species (Hastings 2003; Wimberly 2006). Sedentary species are especially sensitive to decreased

disturbance regimes (Nathan & Muller-Landau 2000; Grau 2002; Warren & Buttner 2007) when usable early successional habitat declines with time. This would not be expected to be an issue for highly vagile species, such as migratory birds, which should be capable of locating and occupying new habitats created through disturbance. Although a number of studies note the adverse affects of disruption of disturbance regimes on migratory birds (e.g. Hunter *et al.* 2001; DeGraaf & Yamasaki 2003; Fink *et al.* 2006), the focus has been on the loss of early successional habitats and not on the issue of colonizing newly available habitat. Models have suggested that even for highly mobile species dependent on early successional habitats, colonization of suitable habitat decreases dramatically as those patches decrease in availability (Wimberly 2006).

For less vagile organisms dependent on early successional habitats, limitations in colonizing ability may have implications for genetic structure (Pannell & Dorken 2006). As

Correspondence: Kelly Barr, Fax: 337-482-5660;

E-mail: kellybarr@gmail.com

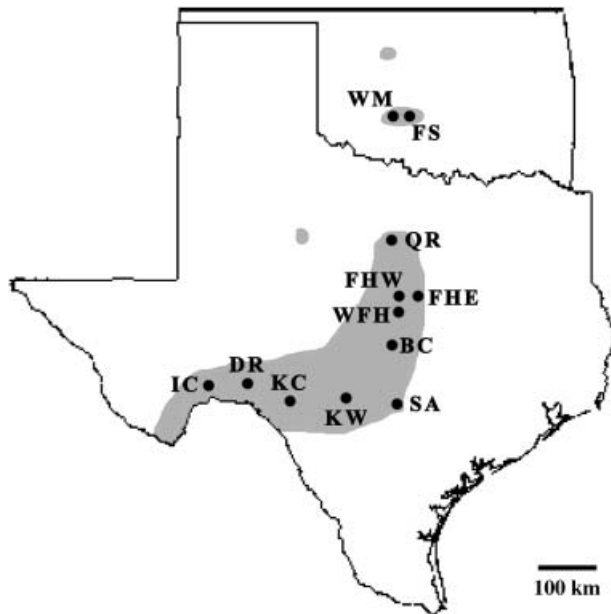


Fig. 1 Approximate locations of sites sampled for black-capped vireos in its breeding range in the USA including Fort Sill (FS), Wichita Mountains Wildlife Refuge (WM), Quail Ridge Ranch (QR), eastern Fort Hood (FHE), western Fort Hood (FHW), West Fort Hood (WFH), Balcones Canyonlands National Wildlife Refuge (BC), a reserve in San Antonio (SA), Kerr Wildlife Management Area (KW), Kickapoo Caverns State Park (KC), Devil's River State Natural Area (DR), and Independence Creek Preserve (IC). See Table 1 for UTM coordinates for these locations. Gray shading indicates the approximate breeding range.

new habitats are created, they are colonized by a limited pool of individuals. This will result in genetic differentiation of newly created habitats due to founder events (Wade & McCauley 1988; Whitlock & McCauley 1990). Theory predicts that this differentiation will decline with time as gene flow has a chance to overcome the effects of drift resulting from the initial colonization event (Pannell & Dorken 2006); in species dependent on early successional communities, however, there may not be sufficient time for gene flow to overcome drift. As a result, genetic structure would be heavily influenced by extinction and colonization events, similar to what might be expected in a classic metapopulation situation with high rates of local extinction (Slatkin 1977). Of course, if movement and gene flow is high among sites, as might be expected in highly vagile organisms, the influence of founder events would be low, and little differentiation should occur among recently colonized sites and more persistent populations.

The endangered black-capped vireo (*Vireo atricapilla*) is a migratory songbird dependent on early successional habitat over much of its range (Grzybowski 1995). Composed of low, dense shrubs, black-capped vireo habitat is often along edge, where disturbance is more common

(Graber 1961; Grzybowski 1995). The principal threats to the species' long-term persistence include increased abundance of a brood parasite, the brown-headed cowbird (*Molothrus ater*), disruption of disturbance regimes due to fire suppression, and conversion of natural habitats to other land uses such as croplands, pastures, and housing developments (Grzybowski 1995; Hunter *et al.* 2002). Each spring, black-capped vireos migrate from their wintering grounds along the Pacific coast of southwestern Mexico to their breeding range narrowly distributed across northern Mexico, central Texas, and southern Oklahoma (Fig. 1). Considering this extensive movement, one would not expect black-capped vireos to have limited dispersal ability or interpopulation genetic differentiation.

Despite its high vagility, there have been a variety of population structures predicted for the species that could result in intersite genetic differentiation, including that of a metapopulation (Grzybowski 1991; Wilkins *et al.* 2006) and a source-sink model (Fazio *et al.* 2004). In their breeding range, adult black-capped vireo males exhibit high site fidelity, with approximately 96% returning annually to previously established territories (Graber 1961; Grzybowski 1995); however, little is known about dispersal between the time of fledging and establishment of breeding territories. An allozyme study using samples collected in 1992 found higher differentiation among the four major extant geographical concentrations of black-capped vireos at the time of the study than is usually seen in songbirds (Fazio *et al.* 2004). Because the study was based on small sample sizes and the results were essentially driven by two loci that may have been biased by selection (Fazio *et al.* 2004), these data are not enough to conclude that black-capped vireo populations are spatially structured. Furthermore, since Fazio *et al.* (2004) conducted their sampling in 1992, black-capped vireo populations have dramatically increased (Wilkins *et al.* 2006). The total US population was thought to be about 180 breeding pairs in 1987, when the species was listed for protection. With protection and active management, population estimates had increased to approximately 1800 singing males by 1995, and to at least 6000 singing males by 2005 (Wilkins *et al.* 2006). Estimates for the breeding population in Mexico are uncertain, but it may be as large as the current US population (Wilkins *et al.* 2006) and the population history is relatively unknown.

If the black-capped vireo were historically a panmictic species, with the differentiation detected by Fazio *et al.* (2004) being an artefact of population bottlenecks and isolation before their protection, genetic structure should have been lost during the recent population expansion. Alternatively, if vireo populations at sites are transitory, and colonization is more important than subsequent gene flow in determining genetic structure, genetic differentiation might persist in the face of overall increases in population

Table 1 Summary of sample sizes (n), average numbers of alleles per locus (A), allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) based on the average of 15 polymorphic microsatellite loci for 12 sampled black-capped vireo populations

| Sample Population | UTM Coordinates* | | Censed Males | n | A | A_R | H_E | H_O | F_{IS} |
|-------------------|------------------|---------|--------------|-----|------|-------|-------|-------|----------|
| | Northing | Easting | | | | | | | |
| WM | 524765 | 3843206 | 1500+ | 17 | 7.40 | 7.17 | 0.76 | 0.73 | -0.006 |
| FS | 536764 | 3839026 | 500+ | 16 | 7.60 | 7.45 | 0.77 | 0.73 | -0.038 |
| QR | 605392 | 3556322 | 27+ | 17 | 7.80 | 7.51 | 0.71 | 0.74 | 0.082 |
| FHW | 607753 | 3440483 | 2400+ | 16 | 7.93 | 7.78 | 0.77 | 0.72 | -0.062 |
| FHE | 613016 | 3466537 | 2400+ | 19 | 7.93 | 7.73 | 0.73 | 0.70 | 0.003 |
| WFH | 601983 | 3127982 | 2400+ | 18 | 8.13 | 7.39 | 0.76 | 0.75 | 0.026 |
| BC | 593627 | 3389776 | 100+ | 17 | 8.07 | 7.81 | 0.78 | 0.77 | 0.030 |
| SA | 531648 | 3274835 | 32+ | 17 | 8.53 | 8.15 | 0.72 | 0.74 | 0.071 |
| KW | 451667 | 3328303 | 435+ | 18 | 8.53 | 8.14 | 0.75 | 0.76 | 0.047 |
| KC | 360754 | 3278084 | 115+ | 16 | 7.93 | 7.66 | 0.76 | 0.74 | -0.011 |
| DR | 310130 | 3312524 | 78+ | 15 | 7.73 | 7.77 | 0.71 | 0.70 | 0.009 |
| IC | 230086 | 3373333 | 60+ | 20 | 8.93 | 8.20 | 0.73 | 0.77 | 0.078 |
| Mean | | | | 17 | 8.04 | 7.73 | 0.74 | 0.74 | 0.019 |

*North American Datum 1983 (NAD83).

†Wilkins *et al.* (2006).

WM, Wichita Mountains Wildlife Refuge; FS, Fort Sill; QR, Quail Ridge Ranch; FHW, western Fort Hood; FHE, eastern Fort Hood; WFH, West Fort Hood; BC, Balcones Canyonlands National Wildlife Refuge; SA, a reserve in San Antonio; KW, Kerr Wildlife Management Area; KC, Kickapoo Caverns State Park; DR, Devil's River State Natural Area; IC, Independence Creek Preserve.

size. Here, we employ highly polymorphic nuclear microsatellites and an extensive sampling regime to ascertain the nature of population structure and genetic diversity in the black-capped vireo's breeding range in Texas and Oklahoma after its recent population expansion. Our objective is to assess the black-capped vireo's population structure, and, if population differentiation and decreased genetic diversity were detected, to determine the underlying mechanisms causing that structure. This work will provide insights into the influence of colonization on the genetic structure of a disturbance-dependent species as well as on the role of population expansion on intersite genetic differentiation in a recovering endangered species.

Materials and methods

Sample collection

Black-capped vireos were sampled at 12 individual sites spread across the species' breeding range in the USA (Fig. 1, Table 1). These sites represent most of the known major populations of black-capped vireos in Texas and Oklahoma (Wilkins *et al.* 2006).

At each site, 15–20 birds were sampled (Table 1). Birds were captured via standard mist netting techniques with song playback. Each bird was sampled for blood by pricking the brachial vein with a sterile needle and collecting 2–3 μ L of blood with a capillary tube. Blood was stored in a

1.5 mL Eppendorf tube containing Puregene cell lysis solution (Gentra Systems). Samples were stored at 4 °C until DNA was extracted. Each bird was banded with a US Fish & Wildlife silver leg-band, aged, and sexed.

DNA extraction and fragment analysis

DNA was extracted with the PureGene DNA Isolation Kit (Gentra Systems). Samples were genotyped at 15 microsatellite loci, including 12 isolated in the black-capped vireo (Barr *et al.* 2007) and three previously identified from the Mariana crow (*Corvus kubaryi*), 2A5A, 1B5D, and 4B6D (Tarr & Fleischer 1998). Polymerase chain reaction conditions for loci developed for black-capped vireos were conducted following Barr *et al.* (2007), but with an annealing temperature of 50 °C for each of the loci from Tarr & Fleischer (1998). One locus described in Barr *et al.* (2007), BCV5-1, was excluded from this study due to amplification inconsistencies. We assessed deviations from Hardy–Weinberg Equilibrium (HWE) and the presence of null alleles using MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004). We tested loci for linkage disequilibrium in GENEPOP 1.2 (Raymond & Rousset 1995).

Spatial structure

We utilized several approaches to evaluate population structure. We estimated F_{ST} (Weir & Cockerham 1984) among

sampling locations and between pairs of locations using GENEPOP 1.2. This program was also used to test for differences in allele frequencies between pairs of sample locations.

To visualize genetic differences among sample sites, Cavalli-Sforza and Edwards chord distance was estimated in MSA 4.05 (Dieringer & Schlotterer 2003), and analysed via nonmetric multidimensional scaling (NMS) in PC-ORD 4.0 (McCune & Mefford 1999). Cavalli-Sforza and Edwards chord distance has been shown to perform better than other such distance measures when using microsatellite markers if the mutational model is not fully known (Takezaki & Nei 1996).

A multitude of clustering approaches are currently available to assess population structure, with little agreement in the literature over which is the most appropriate. We employed several, discussed here in order of their increasing ability to detect population structure among simulated populations with small values of F_{ST} . STRUCTURE 2.2 (Pritchard *et al.* 2000) is the most commonly used method, but lacks the ability to correctly identify population structure when F_{ST} is small (Latch *et al.* 2006; Waples & Gaggiotti 2006; Chen *et al.* 2007). In STRUCTURE, the admixture model was used to analyse $K = 1-24$, with five runs at each K , 500 000 replicates, and a 50 000 replicate burn-in. Default values were maintained for all other parameters. BAPS 3.1 (Corander *et al.* 2003; Corander *et al.* 2005; Corander *et al.* 2006) is a Bayesian assignment approach that has been shown to be more capable than STRUCTURE of correctly assigning individuals when F_{ST} is small (Latch *et al.* 2006). Considering clusters to be those with more than three individuals, as per Corander *et al.* (2005), we estimated clusters in BAPS considering maximum $K = 3, 5, 10$, and 15, with five repetitions at each maximum K . TESS 1.1 is a maximum likelihood-based assignment test that utilizes a priori geographical information and has been shown to perform the best of the three assignment methods we present here when F_{ST} is small (Chen *et al.* 2007). We estimated clusters in TESS utilizing the admixture model, considering a $\psi = 0.6$ and 100 runs with 100 000 sweeps, a 20 000 sweep burn-in, and the default values for the allele frequency model and admixture model parameters. The top 10 highest likelihood runs from TESS (O. Francois, personal communication) were then analysed in CLUMPP 1.0 (Jakobsson & Rosenberg 2007) to correct for between-run discrepancies (e.g. label-switching) common to cluster analyses. The results of CLUMPP were then visualized in DISTRUCT 1.0 (Rosenberg 2004).

We also used a modified contingency test to define populations, as per Waples & Gaggiotti (2006). This method was implemented using GENEPOP 1.2 and the ad hoc statistical criteria suggested by Waples & Gaggiotti (2006). In simulations, this method was much more powerful than assignment tests at identifying the underlying population

structure when F_{ST} values were minimal (0.01–0.02) (Waples & Gaggiotti 2006). In addition, Waples & Gaggiotti (2006) showed that type I error is not problematic in this method, as it rarely identified population structure where none existed.

Geographical analyses

Mantel tests were conducted to analyse the potential effects of geographical distance and the composition of the intervening landscape on genetic differentiation among sample sites. Following Rousset (1997), genetic differentiation was defined as $F_{ST}/(1 - F_{ST})$ for the assessment of isolation by distance (IBD). We evaluated the relationship of genetic differentiation with both linear distance and the logarithm of distance, the latter having been shown by Rousset (1997) to perform better when testing for IBD in a two-dimensional space. In addition to geographical distance, we also examined the association between genetic differentiation and the resistance measure proposed by McRae & Beier (2007).

To assess habitat connectivity, we obtained habitat data from the 2001 National Land-Cover Database (NLCD) (Homer *et al.* 2004) with a spatial resolution of 30 m, and performed analyses with FRAGSTATS 3.0 (McGarigal *et al.* 2002). Because black-capped vireo habitat is difficult to qualify in the geographic information systems (GIS) context, we used a very coarse measure of connectivity, the percentage of the area between a pair of sites containing potentially viable habitat. We considered all agriculture, barren land, urban area, forest interior, and water to be unusable for movement by black-capped vireos. Forest interior was defined as those pixels not being within a pixel-width (30 m) of forest edge, where forest borders scrubland. These between-site indices were calculated for both a 10-km and 20-km wide band around a line connecting the centre points of each site. To account for associations with the percentage of potentially usable habitat and genetic differentiation that might arise due to geographical distance, we used a partial Mantel test to assess the association while holding the influence of geographical distance constant (Smouse *et al.* 1986), recognizing there is debate over whether the test produces biased estimates of P values (Raufaste & Rousset 2001; Castellano & Balletto 2002; Rousset 2002).

Genetic diversity and bottlenecks

Genetic diversity for each sample site was characterized by calculating the mean number of alleles per locus, expected and observed heterozygosities, and F_{IS} values in GENALEX 6.0 (Peakall & Smouse 2006). Allelic richness, the number of alleles adjusted for differences in the numbers of individuals sampled per site, was calculated in FSTAT

Table 2 Genetic differentiation between sites sampled for black-capped vireos. Pairwise F_{ST} values are presented below the diagonal and P values above the diagonal. Emboldened values are significantly differentiated at $\alpha < 0.05$ following a modified Bonferroni adjustment for multiple comparisons (Rice 1989). Overall $F_{ST} = 0.021$ ($P < 0.001$)

| | WM | FS | QR | FHW | FHE | WFH | BC | SA | KW | KC | DR | IC |
|-----|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----------|
| WM | | 0.2739 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| FS | 0.000 | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0009 | < 0.0001 | < 0.0001 |
| QR | 0.037 | 0.034 | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| FHW | 0.031 | 0.037 | 0.029 | | 0.1604 | 0.0052 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0013 |
| FHE | 0.030 | 0.034 | 0.025 | 0.007 | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| WFH | 0.021 | 0.016 | 0.024 | 0.013 | 0.024 | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0003 |
| BC | 0.026 | 0.026 | 0.023 | 0.023 | 0.036 | 0.016 | | < 0.0001 | < 0.0001 | < 0.0001 | 0.0001 | < 0.0001 |
| SA | 0.021 | 0.019 | 0.017 | 0.030 | 0.019 | 0.021 | 0.022 | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| KW | 0.017 | 0.021 | 0.026 | 0.026 | 0.031 | 0.018 | 0.013 | 0.017 | | 0.0010 | 0.0002 | 0.0004 |
| KC | 0.017 | 0.014 | 0.025 | 0.031 | 0.027 | 0.020 | 0.017 | 0.019 | 0.010 | | 0.0138 | 0.0019 |
| DR | 0.032 | 0.028 | 0.059 | 0.049 | 0.053 | 0.038 | 0.016 | 0.039 | 0.017 | 0.013 | | < 0.0001 |
| IC | 0.011 | 0.012 | 0.023 | 0.017 | 0.021 | 0.009 | 0.013 | 0.020 | 0.012 | 0.014 | 0.028 | |

WM, Wichita Mountains Wildlife Refuge; FS, Fort Sill; QR, Quail Ridge Ranch; FHW, western Fort Hood; FHE, eastern Fort Hood; WFH, West Fort Hood; BC, Balcones Canyonlands National Wildlife Refuge; SA, a reserve in San Antonio; KW, Kerr Wildlife Management Area; KC, Kickapoo Caverns State Park; DR, Devil's River State Natural Area; IC, Independence Creek Preserve.

2.9.3.2 (Goudet 1995). F_{STAT} uses rarefaction, a technique shown to perform better than other available methods for estimating allelic richness (Leberg 2002). Differences in heterozygosity and allelic richness were evaluated among sites using PROC GLM in SAS (SAS Institute 2005), using an analysis of variance (ANOVA) that treated each locus as a randomized block to control for interlocus variation.

We assessed the bottleneck history of these populations using BOTTLENECK (Cornuet & Luikart 1996). This program was used to detect a heterozygote excess for individual populations, considering the two-phased model (TPM) of microsatellite mutation, a 70% stepwise-mutation model (SMM) and 30% infinite alleles model (IAM), and 1000 replications. Several other combinations of the SMM:IAM ratio were tested to establish the sensitivity of these data to the mutational mechanism. The Wilcoxon signed-rank test was used to determine if the allele frequency distribution for a population exhibited significant heterozygote excess relative to model expectations. We also examined the ratio of total alleles to the range in allele sizes, or M , another test for a historic bottleneck in individual populations using the program M_{P_VAL} (Garza & Williamson 2001). For these analyses, we used the approach of Waples & Gaggiotti (2006) to identify sets of sites sufficiently differentiated enough to be considered populations, rather than arbitrarily assuming each sample site was an individual population.

Results

We detected significant levels of null alleles in one locus, BCV4-6, at three sample sites; consequently this locus was excluded from further analyses. Evidence for the presence

of null alleles was detected in two other loci, BCV2-5 and BCV4-3, each at different individual sample sites. Considering the 192 tests for null alleles that were conducted, we attributed the detection of nulls in these two comparisons to be within the range expected with a type I error rate of 0.05. Therefore, we did not remove either of these two loci from subsequent analyses. None of the tests for deviation from HWE or for linkage disequilibrium were significant following a sequential Bonferroni adjustment of α .

Spatial structure

Overall F_{ST} among sampled sites was 0.021, with pairwise values ranging from 0 [Wichita Mountains Wildlife Refuge (WM) and Fort Sill (FS)] to 0.053 [Devil's River State Natural Area (DR) and eastern Fort Hood (FHE)] (Table 2). Excepting the differentiation between WM and FS, and western Fort Hood (FHW) and FHE, all tests for pairwise differentiation were significant based on a sequential Bonferroni adjustment of alpha (α) (Table 2; Rice 1989). Because female songbirds often disperse at higher rates than males (Gill 2006) and because male black-capped vireos are highly philopatric (Graber 1961; Gryzbowski 1995), we also evaluated F_{ST} by sex. Restriction of the analysis to males ($n = 170$), did not increase our estimate of overall F_{ST} (0.018). Unfortunately, we were unable to sample a sufficient number of females ($n = 36$) for an analysis restricted to this sex.

NMS ordination of chord distances revealed that the data are best represented in three dimensions (3D), with 8% stress (Fig. 2). Stress represents distortion of relationships between samples resulting from the attempt to

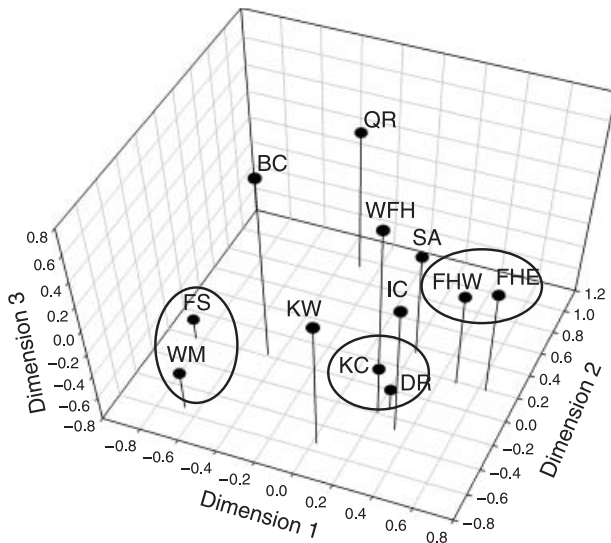


Fig. 2 Results of NMS ordination on chord distance (stress = 8%) among black-capped vireo sample sites. Sites considered to be single populations by the Waples & Gaggiotti (2006) method are circled.

represent the data in multiple dimensions. With less than 10% stress (Kruskal 1964), it can be said that the 3-dimensional depiction is an accurate representation of the relationships among sites based on chord distance. The relationships among sample locations in Fig. 2 are consistent with the pairwise F_{ST} values (Table 2). For instance, the pairs of sites found not to be significantly differentiated, namely WM and FS, and FHW and FHE, are represented relatively proximate in scaled genetic space (Fig. 2). Both the Quail Ridge Ranch (QR) and Balcones Canyonlands National Wildlife Refuge (BC) sites are relatively differentiated from all other sites (Fig. 2). Finally, except for the aforementioned genetically similar sites, there is no notable correlation between physical proximity of sites and their placement in scaled genetic space (Fig. 2).

The assignment test in STRUCTURE 2.2 detected only a single cluster, suggesting that there was no subdivision among our samples. Evidence for two clusters, however, was detected in BAPS 3.1, with 10 individuals representing

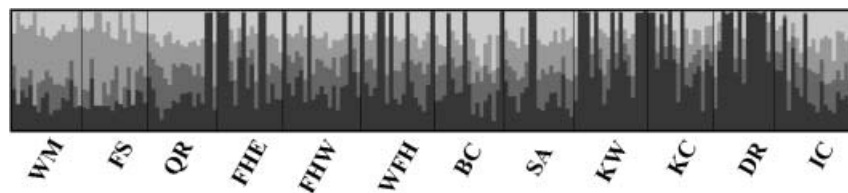


Fig. 3 Results of admixture analysis in TESS for all sites sampled for black-capped vireos. Columns represent cluster membership coefficients for individual birds averaged across the 10 highest likelihood runs. Column shading represents assignment probabilities of birds to individual clusters. Vertical black bars separate sampling sites.

DR and Kickapoo Caverns State Park (KC) being placed in one cluster and all other individuals into another. This result is consistent with the evidence for a close genetic relationship between DR and KC indicated by the genetic distance measures (Fig. 2 and Table 2). The results of admixture analysis in TESS 1.1, portrayed in Fig. 3, provide evidence for sharp differentiation between the Texas and Oklahoma sites. There is also some evidence that DR, KC, and Kerr Wildlife Management Area (KW) represent an individual cluster (Fig. 3).

Several pairs of sample sites were determined to be members of the same population following the method of Waples & Gaggiotti (2006). These included the combinations of FS and WM, FHE and FHW, and DR and KC (Fig. 2). All the remaining sample sites were differentiated extensively enough to be considered individual populations.

Geographical analyses

Analysis of untransformed geographical distance did not detect IBD (Fig. 4; $r = 0.144$, $P = 0.125$); however, analysis of the logarithm transformation of distance reveals a significant relationship with genetic differentiation (Fig. 4; $r = 0.284$, $P = 0.006$). There was no association between genetic distance and resistance ($r = 0.088$, $P = 0.477$), as defined by McRae & Beier (2007).

A positive correlation was detected between the 10-km and 20-km bands for our approximation of connectivity ($r = 0.997$, $P < 0.0001$). For this reason, only data from the 10-km bands are considered for further analyses. There were no significant correlations between habitat and genetic differentiation ($r = -0.099$; $P = 0.212$) or habitat and genetic differentiation with (ln) geographical distance held constant ($r = 0.006$; $P = 0.510$).

Genetic diversity and bottlenecks

There were no detectable differences in heterozygosity ($F = 0.74$, $P = 0.698$) or allelic richness ($F = 0.86$, $P = 0.584$) in comparisons of sample sites. With a Bonferroni adjustment of α , heterozygote excess, which is associated with past bottlenecks, was detected at a single site, BC ($P = 0.004$). None of the other individual sets of genetically

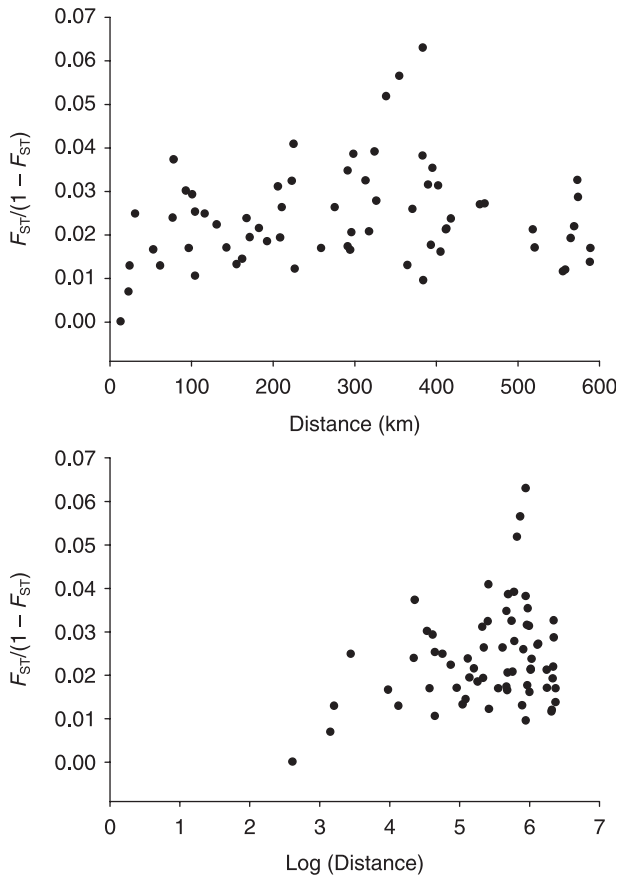


Fig. 4 Relationship of genetic differentiation ($F_{ST}/1 - F_{ST}$) with geographical distance (top) and the logarithm of geographical distance (bottom).

similar sites displayed significant heterozygote excess. M -ratios were all > 0.76 , which is substantially higher than the threshold of ≤ 0.68 expected for a bottlenecked population (Garza & Williamson 2001).

Discussion

Spatial structure

Population differentiation is prevalent throughout the range of the black-capped vireo, with all pairs of sample sites other than those located very close together (< 20 km) being significantly differentiated (Table 2). To date, no other microsatellite-based population studies have been conducted on other vireos, so we cannot compare this level of differentiation to closely related species. Many previous studies of migratory songbirds did not detect significant genetic differentiation between pairs of sites separated by 50–100 km (e.g. Arguedas & Parker 2000; Gibbs *et al.* 2000; Clegg *et al.* 2003; Veit *et al.* 2005). Therefore, our results support the conclusion of Fazio *et al.* (2004)

that genetic structure in this species is high compared to other songbirds.

The differentiation among black-capped vireo sample sites is substantially higher than that observed among sample sites of another endangered songbird, the golden-cheeked warbler (*Dendroica chrysoparia*), that occurs in many of the same sample sites. If only the sites within the golden-cheeked warbler's range are considered [excluding WM, FS and Independence Creek Preserve (IC)], the overall F_{ST} for the black-capped vireo, 0.0174, is double that of the golden-cheeked warbler, 0.008 (Lindsay *et al.* 2008). Despite the fact that their occupied habitat is often contiguous with that of the black-capped vireo, golden-cheeked warblers have very different requirements. Whereas the former is found in dense scrub characterized by early successional vegetation, the latter is found in late-successional ashe juniper (*Juniperus ashei*) mixed with hardwoods (Ladd & Gass 1999). Golden-cheeked warblers also require large patches of habitat, while black-capped vireos are often found along edges and in small stands, or mottes (Gryzbowski 1995). Even with the ability to inhabit edge and smaller patches (which are much more available than large, continuous blocks of habitat), black-capped vireos exhibit far greater genetic differentiation than does the golden-cheeked warbler. This suggests that gene flow might be lower in black-capped vireos, which need early successional habitats, than in the late-successional specialist golden-cheeked warbler. Considering that golden-cheeked warblers have not experienced the dramatic population recovery in numbers that has occurred in the black-capped vireo, this finding was unexpected.

At relatively low values of F_{ST} , BAPS and TESS have been shown to have more power at detecting population subdivision than has STRUCTURE (Latch *et al.* 2006; Chen *et al.* 2007). Our results reflect these known differences in power. While STRUCTURE did not resolve any clusters, BAPS provided evidence for two clusters and TESS for three clusters. The TESS results seem to concur most closely with those based on genetic differentiation among sample sites. These results indicate that the Oklahoma populations differ considerably from those in Texas, and that there is some clustering of KW, KC, and DR (Fig. 3). The Waples & Gaggiotti (2006) approach identified nine populations among our 12 sites, including both the KC and DR cluster identified in BAPS and the FS and WM cluster detected in TESS. This approach also detected an association between FHE and FHW (Fig. 2). While the members of FHE and FHW, and FS and WM are separated by < 20 km, DR and KC are separated by approximately 60 km.

In general, we found that sites separated by very short distances and potentially well connected by viable habitat tended to be genetically similar. Abundant habitat known to be inhabited by black-capped vireos exists between both FHE and FHW and between the two Oklahoma

populations. We cannot make this assertion for the DR and KC sites; however, the landscape between the latter sites is largely outside of the influence of anthropogenic activities, and therefore might be more readily used by dispersing birds. While West Fort Hood (WFH) is separated by a similar geographical distance from both FHE and FHW as that separating FHE and FHW, FS and WM, and KC and DR, much of the habitat between WFH and the other Fort Hood sites has been converted to urban area, mostly in the past 50 years.

The amount of genetic differentiation among sites is not consistent with the expectations for a panmictic population of individuals capable of moving between habitat patches as they become available. Under such a model, little genetic differentiation would be expected between sample locations. Instead, our analyses are consistent with a model where gene flow is limited when populations are separated by > 60 km or at shorter distances if there is a lack of suitable intervening habitat.

Geographical analyses

While there is no strong association between genetic differentiation and geographical distance, the relationship was strengthened by using the logarithm of geographical distance (Fig. 4). Genetic differentiation is expected to be more strongly associated with the log of geographical distance than with untransformed distance when gene flow is occurring in two dimensions (Rousset 1997); conversely, a stronger relationship with untransformed distance is expected when populations are arrayed in a linear habitat. The stronger IBD relationship of genetic distance with log distance rather than untransformed distance is somewhat surprising in the case of black capped vireos, which have a relatively linear distribution across Texas and Oklahoma (Fig. 1). Another explanation for the significant relationship between log geographical distance and genetic distance is that the log transformation emphasizes the small distance comparisons while deemphasizing longer distances. This pattern would be expected in a model where gene flow and genetic drift are differentially influential, with gene flow being more influential on small scales and genetic drift at large distances (Hutchinson & Templeton 1999; Koizumi *et al.* 2006). Once distances separating pairs of populations exceed 100 km, there appears to be little evidence that genetic differentiation increases with greater distances (Fig. 4, top).

If black-capped vireos were a formerly panmictic population with limited contemporary gene flow due to habitat fragmentation, it would be expected that both geographical distance and lack of connectivity would lead to increased differentiation among sites. We cannot discount the possibility that our coarse quantification of usable

habitat may not accurately quantify actual connectivity between populations. The ability of the species, however, to use small patches of early successional habitat would lead one to assume that disturbances should generate patches of habitat useful to the vireos. As long as neither land cover has been entirely removed and disturbance processes have not been completely disrupted, such habitat patches should be available for use in dispersal or colonization.

Genetic diversity and bottlenecks

Evidence for a bottleneck was detected in only one population. While this population, BC, is presently composed of a relatively large number of birds (Table 1), there is anecdotal evidence that the area historically supported only a few pairs, likely due to low habitat availability (C. Sexton, personal communication). A population bottleneck might explain the relative isolation of BC in Fig. 2. Furthermore, genetic differentiation between this sample site and other sites was less associated with the log of geographical distance than what was detected in the other sites (data not shown). Combined with the anecdotal evidence for being a historically small population, our analysis may be detecting the signal of a founder event or other bottleneck at BC. We found no evidence of strong bottlenecks in any other population, and levels of allelic richness were not reduced in any of the populations regardless of their current size.

Unfortunately, current statistical tests for bottlenecks only have the power to detect very severe and extended reductions in population size, and this power is further diminished by gene flow (Cornuet & Luikart 1996; Garza & Williamson 2001). Thus, studies sometimes fail to detect genetic signatures of known bottleneck or founder events (Spencer *et al.* 2000; Clegg *et al.* 2002; Busch *et al.* 2007). Although we did not detect evidence of severe bottlenecks, there is still substantial genetic differentiation among many sites. It is possible that high drift occurring after founder events and prolonged small size in some cases [e.g. QR and a reserve in San Antonio (SA)] is sufficient enough to overcome the reduction of genetic differentiation that might otherwise occur when gene flow is high. This would disrupt patterns expected under IBD, but would not necessarily produce a genetic signal of strong bottlenecks. Given the likelihood that drift and gene flow have probably not reached equilibrium in this system, it is not possible to estimate the amount of gene flow between sites; gene flow, however, does not appear to be high enough to view the sampled locations as a single panmictic population.

Many of our sample sites recently supported considerably smaller population sizes of black-capped vireos relative to today, suggesting that bottlenecks have taken

place. We have already mentioned the low historic population size at BC. The population at QR was established in the mid-1990s, and has since been maintained at a relatively low size (R. Fain, personal communication). Anecdotally, the area now encompassed by Fort Hood was nearly barren before the establishment of the installation in 1942, and probably had little black-capped vireo habitat (G. Eckricht, personal communication). In 1991, Fort Hood consisted of several hundred black-capped vireos (Gryzbowski 1991); by 2006, however, the population had swelled to several thousand (Cimprich 2006). During the 1950s, Graber (1961) detected no black-capped vireos in Comanche County, Oklahoma, where FS and WM are located; today, however, there are known to be at least 2400 males at these two sites (Wilkins *et al.* 2006). The patterns seen at these sites are likely representative of the overall pattern across the black-capped vireo's breeding range. Thus, black-capped vireo populations have rebounded considerably since their listing in 1987, mostly due to habitat protection and management, and brown-headed cowbird control (Wilkins *et al.* 2006).

It could be argued that the significant differentiation we detected is a remnant of the extended period of time during which population sizes were highly reduced. If re-established gene flow between populations has not recovered sufficiently enough or for a long enough period of time, rebounding populations may be retaining the signal of past founder events (Hutchinson & Templeton 1999; Koizumi *et al.* 2006). If the lack of gene flow between distantly separately aggregations best explained the differentiation between them, then we would have expected a significant IBD signal in analysis of untransformed distances. That we detected IBD after the log transformation of the distances is indicative that genetic drift is the driving force behind differentiation at higher distances. It is also telling that genetic differentiation occurs between IC and both DR and KC, three sites outside of the range of the brown-headed cowbird where black-capped vireo populations have persisted prior their listing (Gryzbowski 1991). There is no evidence of large changes in habitat availability or population size at these sites, suggesting that even between stable populations, gene flow may not be high enough to overcome the effects of drift on genetic differentiation.

Fazio *et al.* (2004) suggested the WM and FS populations to be parts of a source-sink system, with the Oklahoma sites receiving unidirectional gene flow from larger populations in Texas. This conclusion is based on their findings of high genetic diversity in the WM and FS population, an unanticipated result due to the geographical isolation of WM and FS and the extreme prior attenuation of the population – the known population as of 1991 was approximately 225 individuals (Gryzbowski 1991; Fazio *et al.* 2004). A source-sink dynamic, however, would exhibit signs of high gene flow among groups (Hanski

1999). Although we sampled nearly every extant significant aggregation of black-capped vireos (Wilkins *et al.* 2006), we detected no such relationship. Therefore, we have no evidence to support the model suggested by Fazio *et al.* (2004). It is likely that coupled with some gene flow, the bottlenecked population size of dozens of breeding pairs was insufficient in severity and time to reduce levels of genetic diversity in WM and FS populations significantly.

Conclusion

Our data suggest that black-capped vireos are not moving and dispersing across great distances in large numbers. Even where habitat is abundant, such as in central Texas, genetic differentiation exists between many sites. Considering the migratory songbird's vagility, gene flow would have been expected to be related to the level of connectivity between sites. Furthermore, the rapid expansion of the species across much of its breeding range would suggest gene flow would be very high. The existence of substantial genetic differentiation despite high potential vagility and population expansion argue for greater attention to dispersal behaviour when considering the species' population and genetic dynamics. Unfortunately, little is known about dispersal between the time of fledging and the establishment of breeding territories by adults.

While the species does not currently persist as a metapopulation per se, our finding of locally limited gene flow implies they might be treated as a metapopulation for management purposes. Many sites with large numbers of birds today were small or nonexistent in the recent past, and may have been established with birds from far smaller populations. Small concentrations of breeding birds might still be important for connectedness of larger sites, acting as stepping stones. The maintenance of populations over a large spatial scale is also necessary since much of the black-capped vireo's breeding habitat is ephemeral. Rangelands, like those encompassing most of the distribution of the black-capped vireo, were maintained before European settlement in a spatial-temporal mosaic by fire, natural grazing, and other such disturbances (Fuhlendorf & Smeins 1997; Fuhlendorf & Engle 2001). Patches occupied by black-capped vireos would have eventually been lost to succession, and gene flow would have occurred through the colonization of new patches. In such a system, the genetic differentiation of patches would depend on both the numbers of founding individuals and the number of sources from which founders were drawn. Before the disruption of disturbance regimes and the contemporary management practice of maintaining large areas of homogenous vegetative cover (Fuhlendorf & Engle 2001; Fuhlendorf & Engle 2004), suitable habitat patches were likely available more or less continuously across the range of the species. The effect of the disruption of disturbance

regimes on vireo populations has likely been compounded by the extreme loss of habitat to agriculture and urbanization. Dynamic shifting systems of patches require areas sufficiently large enough to accommodate small-scale disturbances in order to maintain a stable amount of habitat in each successional state (DeAngelis & Waterhouse 1987). Even for highly mobile species, colonization of habitat patches in shifting mosaics decreases dramatically once the portion of landscape that includes suitable habitat falls below 30% (Wimberly 2006), which is certainly the case for black-capped vireos. Because disturbance regimes have been highly altered and natural habitats have been lost from much of its distribution, suitable early succession habitat is only available at the small number of sites where there is active management. Thus, black-capped vireos may be in a transition phase between a metapopulation with gene flow occurring with the colonization of newly created, yet ephemeral habitat patches, to a statically structured set of populations, without much movement over space. Future sampling of these populations will prove invaluable in determining whether our results represent a signature of this transition or a stable equilibrium established through intensive management of this species.

Acknowledgements

We thank the many people who assisted with field sampling and logistics, including G. Ekricht, R. Fain, C. Farquhar, V. Fazio, D. Frels, J. Gryzbowski, S. Hodge, J. Karges, J. Kimball, L. Lapham, E. Leibgold, J. Lindsay, S. Miller, K. Moore, R. Myers, J. Neal, C. Pekins, I. Pollet, D. Riskind, C. Sexton, T. Ransom, G. Wampler, M. Wilcox, and D. Wolf. Access to field sites was kindly provided by the US Army, US Fish and Wildlife Service, Texas Parks and Wildlife Department, The Nature Conservancy, Quail Ridge Ranch, Panther Cave Ranch, Dobbs Run Ranch, and Environmental Defense. This research was funded by the Environmental Quality and Installations Thrust Area of the US Army Basic Research Program, with additional support from the University of Louisiana's Graduate Student Organization.

References

- Arguedas N, Parker PG (2000) Seasonal migration and genetic population structure in house wrens. *The Condor*, **102**, 517–528.
- Barr KR, Dharmarjan G, Rhodes OE, Lance RF, Leberg PL (2007) Novel microsatellite loci for the study of the black-capped vireo (*Vireo atricapillus*). *Molecular Ecology Notes*, **7**, 1067–1069.
- Busch JD, Waser PM, DeWoody JA (2007) Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). *Molecular Ecology*, **16**, 2450–2462.
- Castellano S, Balleto E (2002) Is the partial Mantel test adequate? *Evolution*, **56**, 1871–1873.
- Chen C, Durand E, Forbes F, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, **7**, 747–756.
- Cimprich DA (2006) Monitoring of the black-capped vireo during 2006 on Fort Hood, Texas. In: *Endangered Species Monitoring and Management at Fort Hood, Texas: 2006 Annual Report*. The Nature Conservancy, Fort Hood Project, Fort Hood, Texas.
- Clegg SM, Degnan SM, Kikkawa J, Moritz C, Estoup A, Owens IPF (2002) Genetic consequences of sequential founder events by an island-colonizing bird. *Proceedings of the National Academy of Sciences, USA*, **99**, 8127–8132.
- Clegg SM, Kelly JF, Kimura M, Smith TB (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Molecular Ecology*, **12**, 819–830.
- Corander J, Walmann P, Sillanpaa MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367–374.
- Corander J, Walmann P, Marttinen P, Sillanpaa MJ (2004) *VARP* 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, **20**, 2363–2369.
- Corander J, Marttinen P, Mantyniemi S (2005) Bayesian identification of stock mixtures from molecular marker data. *Fisheries Bulletin*, **104**, 550–558.
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.
- DeAngelis DL, Waterhouse JC (1987) Equilibrium and nonequilibrium concepts in ecological models. *Ecological Monographs*, **57**, 1–21.
- DeGraaf RM, Yamasaki M (2003) Options for managing early-successional forest and shrubland bird habitats in the northeastern United States. *Forest Ecology and Management*, **185**, 179–191.
- Dieringer D, Schlotterer C (2003) Microsatellite analyser (*MSA*): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, **3**, 167–169.
- Fazio VW III, Miles DB, White MM (2004) Genetic differentiation in the endangered black-capped vireo. *Condor*, **106**, 377–385.
- Fink AD, Thompson FR III, Tudor AA (2006) Songbird use of regenerating forest, glade and edge habitat types. *Journal of Wildlife Management*, **70**, 180–188.
- Fuhlendorf SD, Engle DM (2001) Restoring heterogeneity on rangelands: ecosystem management based on evolutionary grazing patterns. *Bioscience*, **51**, 625–632.
- Fuhlendorf SD, Engle DM (2004) Application of the fire–grazing interaction to restore a shifting mosaic on tallgrass prairie. *Journal of Applied Ecology*, **41**, 604–614.
- Fuhlendorf SD, Smeins FE (1997) Long-term vegetation dynamics mediated by herbivores, weather and fire in a *Juniperus quercus* savanna. *Journal of Vegetation Science*, **8**, 819–828.
- Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.
- Gibbs HL, Dawson RJG, Hobson KA (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137–2147.
- Gill FB (2006) *Ornithology*. W.H. Freeman Co., New York.
- Goudet J (1995) *FSAT* version 1.2: a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.
- Graber JW (1961) Distribution, habitat requirements, and life

- history of the black-capped vireo (*Vireo atricapilla*). *Ecological Monographs*, **31**, 313–336.
- Grau HR (2002) Scale-dependent relationships between treefalls and species richness in a Neotropical montane forest. *Ecology*, **83**, 2591–2601.
- Grzybowski J (1991) *Black-Capped Vireo (Vireo atricapillus) Recovery Plan*. US Fish and Wildlife Service, Albuquerque, New Mexico.
- Grzybowski J (1995) Black-capped Vireo (*Vireo atricapillus*). In: *The Birds of North America Online* (ed. Poole A). Cornell Laboratory of Ornithology, Ithaca, New York.
- Hanski I (1999) *Metapopulation Ecology*. Oxford University Press, New York.
- Hastings A (2003) Metapopulation persistence with age-dependent disturbance or succession. *Science*, **301**, 1525–1526.
- Homer C, Huang C, Yang L, Wylie B, Coan M (2004) Development of a 2001 national land-cover database for the United States. *Photogrammetric Engineering and Remote Sensing*, **70**, 829–840.
- Hunter WC, Buehler DA, Canterbury RA, Confer JL, Hamel PB (2002) Conservation of disturbance-dependent birds in eastern North America. *Wildlife Society Bulletin*, **29**, 440–455.
- Hutchinson DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative rates of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.
- Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.
- Koizumi I, Yamamoto S, Maekawa K (2006) Decomposed pairwise regression analysis of genetic and geographic distance reveals a metapopulation structure of stream-dwelling Dolly Varden charr. *Molecular Ecology*, **15**, 3175–3189.
- Kruskal JB (1964) Nonmetric multidimensional scaling: a numerical method. *Psychometrika*, **29**, 115–129.
- Ladd C, Gass L (1999) Golden-cheeked warbler: *Dendroica chrysoparia*. In: *The Birds of North America* (eds Poole A, Gill F), Vol. 420, pp. 1–23. Cornell Laboratory of Ornithology, New York, and the Academy of Natural Sciences, Philadelphia.
- Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE (2006) Relative performances of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295–302.
- Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, **11**, 2445–2449.
- Lindsay DL, Barr KR, Lance RF, Tweddle SA, Hayden TJ, Leberg PL (2008) Habitat fragmentation and genetic diversity of an endangered, migratory songbird, the golden-cheeked warbler (*Dendroica chrysoparia*). *Molecular Ecology*, **17**, 2122–2133.
- Luikart G, Cornuet JM (1996) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, **12**, 228–237.
- McCune B, Mefford MJ (1999) *Multivariate Analysis of Ecological Data*, Version 4.26. MjM Software Design, Gleneden Beach, Oregon.
- McGarigal K, Cushman SA, Neel MC, Ene E (2002) FRAGSTATS: spatial pattern analysis program for quantifying landscape structure. University of Massachusetts, Amherst, Massachusetts.
- McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences, USA*, **104**, 19885–19890.
- Nathan R, Muller-Landau H (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution*, **15**, 278–285.
- Neill C (2007) The challenge of managing disturbance regimes, terrestrial communities and rare species in a suburbanizing region: the northeastern US coastal sand plain. *Biological Conservation*, **136**, 1–3.
- van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- Pannell JR, Dorken ME (2006) Colonisation as a common denominator in plant metapopulations and range expansions: effects on genetic diversity and sexual systems. *Landscape Ecology*, **21**, 837–848.
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Raufaste N, Rousset F (2001) Are partial mantel tests adequate? *Evolution*, **55**, 1703–1705.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenism. *Journal of Heredity*, **86**, 248–249.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.
- Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.
- Rousset F (2002) Partial Mantel tests: reply to Castellano and Bolleto. *Evolution*, **56**, 1874–1875.
- SAS Institute (2005) SAS Onlinedoc 9.1.3. SAS Institute Inc., Cary, North Carolina.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.
- Spencer CS, Neigel J, Leberg PL (2000) Evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology*, **9**, 1517–1528.
- Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, **144**, 389–399.
- Tarr CL, Fleischer RC (1998) Primers for polymorphic GT microsatellites isolated from the Mariana crow (*Corvus kubaryi*). *Molecular Ecology*, **7**, 247–255.
- Veit ML, Robertson RJ, Hamel PB, Friesen VL (2005) Population genetic structure and dispersal across a fragmented landscape in cerulean warblers (*Dendroica cerulea*). *Journal of Conservation Genetics*, **6**, 159–174.
- Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.
- Waples RS, Gaggiotti O (2006) What is a population? An empirical study of some genetic methods for identifying the number of

- gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.
- Warren SD, Buttner R (2007) Active military training areas as refugia for disturbance-dependent endangered species. *Journal of Insect Conservation*, doi: 10.1007/S10841-007-9109-2.
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Whitlock MC, McCauley DE (1990) Some genetic consequences of colony formation and extinctions: genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.
- Wilkins N, Powell RA, Conkey AAT, Snelgrove AG (2006) Population status and threat analysis for the black-capped vireo. US Fish and Wildlife Service, Region 2, Albuquerque, New Mexico.
- Wimberly MC (2006) Species dynamics in disturbed landscapes: when does a shifting habitat mosaic enhance connectivity? *Landscape Ecology*, **21**, 35–46.

Kelly Barr has interests in conserving biodiversity and in using molecular tools to study the consequences of human activities on the natural world. This study was the product of his masters thesis. Giri Athrey and Paul Leberg of the University of Louisiana and Richard Lance and Denise Lindsay of the U.S. Army Engineer Research and Development Center Environmental Laboratory share interests in population and conservation genetics. Scott Tweddle and Timothy Hayden of the U.S. Army Engineer Research and Development Center Construction Engineering Research Laboratory have interests, respectively, in landscape modeling and threatened and endangered species conservation.
