

Habitat fragmentation and genetic diversity of an endangered, migratory songbird, the golden-cheeked warbler (*Dendroica chrysoparia*)

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Abstract

Landscape genetic approaches offer the promise of increasing our understanding of the influence of habitat features on genetic structure. We assessed the genetic diversity of the endangered golden-cheeked warbler (*Dendroica chrysoparia*) across their breeding range in central Texas and evaluated the role of habitat loss and fragmentation in shaping the population structure of the species. We determined genotypes across nine microsatellite loci of 109 individuals from seven sites representing the major breeding concentrations of the species. No evidence of a recent population bottleneck was found. Differences in allele frequencies were highly significant among sites. The sampled sites do not appear to represent isolated lineages requiring protection as separate management units, although the amount of current gene flow is insufficient to prevent genetic differentiation. Measures of genetic differentiation were negatively associated with habitat connectivity and the percentage of forest cover between sites, and positively associated with geographic distance and the percentage of agricultural land between sites. The northernmost site was the most genetically differentiated and was isolated from other sites by agricultural lands. Fragmentation of breeding habitat may represent barriers to dispersal of birds which would pose no barrier to movement during other activities such as migration.

Keywords: connectivity, genetic distance, habitat specialist, microsatellite, passerine, population structure

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Introduction

Fragmentation may decrease connectivity and increase distance between remnant habitat patches. In the case of threatened and endangered avian species, the risk of extinction due to the effects of fragmentation is greater because they usually exist at low densities (Saunders *et al.* 1991; Johnson 2001). Smaller, more isolated patches of habitat are characterized by a decreased likelihood of males attracting females and less successful dispersal of offspring (Rappole *et al.* 2003). Decreased dispersal can lead to a

more restricted distribution and further reduce abundance (Villard *et al.* 1999). Moreover, as distance between patches of habitat increases, recolonization of vacated patches becomes more difficult and the ability of breeders to move among populations decreases (Wiens 1994).

The fragmentation of habitat and consequent reduction in population size and gene flow could result in increased loss of genetic diversity within populations and increased genetic differentiation among populations (Frankham 1995). Few investigations of avian populations have reported an association between anthropogenic habitat fragmentation and reduced gene flow leading to increased differentiation. Most studies that have found an association between fragmented populations, and genetic differentiation have involved relatively sedentary gallinaceous birds or

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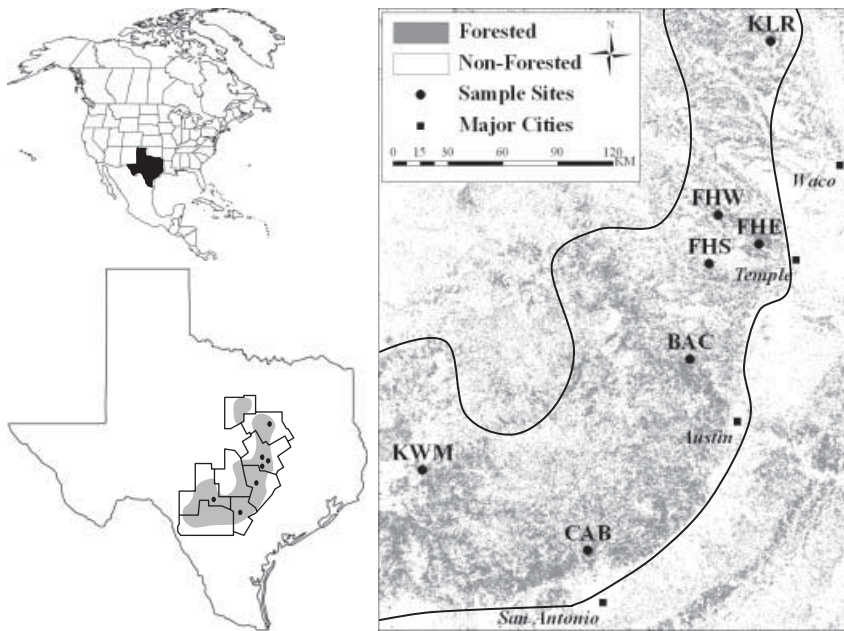


Fig. 1 Map of sample site locations (dots), recovery units (polygons) and the current breeding range (shaded) of the golden-cheeked warbler in Texas (adapted from USFWS 2004), with a map of North America for reference. The expanded map depicts forested and nonforested landcover (2001 National Land-cover Database) in the vicinity of sampling locations, with an outline of the golden-cheeked warbler breeding range and nearby cities for reference. Sample sites included the Klondike Ranch (KLR), Fort Hood Military Reservation West (FHW), Fort Hood Military Reservation East (FHE), Fort Hood Military Reservation South (FHS), Balcones Canyonlands National Wildlife Refuge (BAC), Camp Bullis Training Site (CAB) and Kerr Wildlife Management Area (KWM).

ratites (Leberg 1991; Bouzat 2001; Caizergues *et al.* 2003); although an apparent effect of anthropogenic habitat fragmentation on genetic structure has been observed in sedentary passerines (Brown *et al.* 2004). For highly mobile avian species, such as many songbirds or woodpeckers, most studies have not detected an association between the level of genetic differentiation and anthropogenic habitat fragmentation (Ellegren *et al.* 1999; Bates 2000; Galbusera *et al.* 2004; Veit *et al.* 2005). It is not clear whether the lack of fragmentation effects on genetic structure in this latter group is due to their higher mobility, the recent nature of much of the habitat loss or the small number of taxa that have been studied.

One such mobile species subject to the potential genetic consequences of habitat fragmentation is the golden-cheeked warbler (*Dendroica chrysoparia*), a migratory songbird that winters in Central America and breeds in a range restricted to central Texas (Fig. 1). Between 1962 and 1990, the estimated number of golden-cheeked warbler breeding territories declined from 18 500 to 13 800, corresponding to a 35% reduction in breeding habitat, leading to the listing of the species as endangered in 1990 (USFWS 1992). Today, the breeding habitat of the species is highly fragmented throughout its range. A nesting-habitat specialist, the golden-cheeked warbler has a breeding range limited to dense, mature stands of Ashe juniper (*Juniperus ashei*) mixed with deciduous trees (mostly *Quercus* spp.). Banding data suggests that golden-cheeked warblers are highly philopatric (Ladd & Gass 1999), and their reproduction appears to be adversely affected by small patch sizes and increased edge habitat (Wahl *et al.* 1990; Ladd & Gass 1999). The tendency toward philopatry and dependence on large

patches of mature forests contributes to this species' potential sensitivity to habitat fragmentation.

Landscape genetics, through integration of genetic and habitat data with spatial analysis tools, offers opportunities to better understand the role of habitat features as dispersal barriers (Storfer *et al.* 2007). Our objectives were to use both traditional and landscape approaches to understand patterns of genetic diversity within and among golden-cheeked warbler populations across their breeding range, and to assess correlations between landscape features and genetic structure. We predicted that habitat fragmentation would lead to reduced gene flow between populations, and, combined with decreased population sizes, would result in reduced allelic diversity and heterozygosity within populations.

Materials and methods

Sample collection

Seven study sites were selected throughout the breeding range of the species (Fig. 1), representing almost all the remaining concentrations of singing males. We lured adults into mist nets using song playback and collected blood samples by pricking the brachial vein with a sterile needle and drawing two drops of blood into a non-heparinized capillary tube. Blood samples were stored in PureGene cell lysis solution (Gentra Systems) at 4 °C.

DNA extraction and analysis

DNA was extracted from blood using the PureGene DNA Isolation Kit Non-Mammalian Whole Blood Protocol (Gentra Systems). Using DNA from a subset of golden-cheeked

warbler samples, we evaluated 20 microsatellite primer pairs developed for the black-throated blue warbler (*Dendroica caerulescens*), yellow warbler (*Dendroica petechia*), Swainson's warbler (*Limnothlypis swainsonii*) and Wilson's warbler (*Wilsonia pusilla*). We identified nine loci for use in this study that were polymorphic, produced consistent results across runs and demonstrated no evidence of null alleles based on MICROCHECKER (van Oosterhout *et al.* 2003). The loci used included *Dca32* from *D. caerulescens* (Chuang-Dobbs *et al.* 2001; Webster *et al.* 2001); *Dpμ05* and *Dpμ16* from *D. petechia* (Dawson *et al.* 1997; Yezerinac *et al.* 1999; Gibbs *et al.* 2000); *Lswμ5B*, *Lswμ7* and *Lswμ18* from *L. swainsonii* (Winker *et al.* 1999); and *WpD4*, *WpD23* and *WpD30* from *W. pusilla* (Clegg *et al.* 2003). We used the polymerase chain reaction (PCR) reagent concentrations provided for each locus in their original publications. While the loci *Dca32*, *Dpμ16*, *Lswμ5B*, *Lswμ7* and *Lswμ18* are dinucleotide, *Dpμ05* is a pentanucleotide and *WpD4*, *WpD23* and *WpD30* are tetranucleotides.

Each PCR was conducted via 35 cycles of 95 °C for 60 s, the annealing temperature for 60 s, and 72 °C for 30 s, with an initial denaturation cycle of 95 °C for 3.5 min and a final incubation of 72 °C for 5 min. Annealing temperatures, optimized for golden-cheeked warblers, were 46 °C for *Dpμ05*, *Dpμ16*, *Lswμ7*, *Lswμ18*, *WpD4*, *WpD23* and *WpD30*, and 48 °C for *Dca32* and *Lswμ5B*. Negative controls were included in all amplifications.

Genotypes were collected on both ABI Prism 310 and 3130 Genetic Analysers (Perkin Elmer) due to availability, with either GENESCAN or GENEMAPPER software (Applied Biosystems) and either TAMRA 500 or ROX 500 size standards. Repetitive genotyping between machines and size standards was conducted to confirm consistency. Fragment analysis reactions consisted of 0.5–1 μL PCR product, 0.5 μL size standard and HiDi Formamide (Applied Biosystems) to 15 μL. All putative homozygotes and a subset of all individuals were amplified and genotyped multiple times to confirm their status.

Genetic diversity

Genetic diversity within sample sites was characterized by calculating the mean number of alleles per locus, expected and observed heterozygosity and F_{IS} values in GENEPOP 3.4 (Raymond & Rousset 1995). We also used GENEPOP to determine whether loci deviated from Hardy–Weinberg Equilibrium (HWE) or exhibited linkage disequilibrium. We calculated allelic richness using rarefaction in FSTAT to correct for sample-size differences among sites (Goudet 2001). We conducted an analysis of variance using PROC GLM (SAS Institute 2005) to determine whether differences in heterozygosity or allelic richness existed among sites, treating each locus as a randomized block to control for interlocus variation (Leberg 1992).

To evaluate whether individuals from sampled sites were from a population that had experienced a recent bottleneck, we used 1000 replicates of the two-phased model (TPM) with 70% of the mutations following a step-wise mutation model (SMM) and 30% following an infinite alleles model (IAM) in the program BOTTLENECK (Cornuet & Luikart 1996). The Wilcoxon sign-rank test was used to determine whether the number of loci with heterozygote excess was significantly different than expected if no bottleneck had occurred. We also used the program M_P_VAL (Garza & Williamson 2001) to calculate the ratio (M) of the number of alleles from a sample site to the total range in allele sizes. Our sample sizes from individual sites were not large enough to ensure that we sampled most alleles at each site, so we compared estimates of M to estimates of M_c obtained using the two-phase model in the program CRITICAL_M (Garza & Williamson 2001). We used the TPM with the conservative parameter estimates recommended by Garza & Williamson (2001). If the observed value of M was in the lower 5% of the distribution of M_c values, it was taken as evidence that the sample was from a population that had experienced a recent bottleneck. We also compared an estimate of M from a combined sample of all individuals to a M_c value of 0.68, where an estimate of $M > 0.68$ provides evidence that the population is unlikely to have experienced a recent, severe reduction in size (Garza & Williamson 2001).

Population structure

Population structure was evaluated with pairwise F_{ST} tests and tests for allele frequency differentiation in GENEPOP 3.4. To further evaluate the genetic structure of the population, we used three different approaches, discussed in order of their increasing ability to detect population structure among simulated populations with small values of F_{ST} . First, we estimated the number of genetically divergent groups using STRUCTURE 2.2 (Pritchard *et al.* 2000), a commonly used Bayesian clustering method. We implemented STRUCTURE using the admixture and correlated allele frequency model with 500 000 replicates of the Markov Chain following a 100 000 replicate burn-in. Default values were maintained for all other parameters. Because the highest log-likelihood values were obtained for $K = 1$ (where K is the number of populations), indicating no detectable population structure, further analyses with this approach are not presented.

Population structure was also assessed using BAPS 5.0 (Corander *et al.* 2003, 2004, 2006), a Bayesian assignment approach that performs better than STRUCTURE when F_{ST} is small (Latch *et al.* 2006). We implemented an admixture analysis following the strategy of Corander & Marttinen (2006). This approach begins with a mixture analysis to identify the number of underlying populations in the data.

Considering clusters to be those with more than three individuals, as per Corander *et al.* (2006), we estimated the number and composition of clusters considering maximum $K = 5, 10$ and 15 , with 20 repetitions at each maximum K . The allele frequencies of genetically divergent groups identified in the mixture analysis were then used to conduct the admixture analysis (Corander & Marttinen 2006). This method avoids difficulties with the simultaneous estimation of number of differentiated ancestral populations and the level of admixture in sampled individuals. Following Corander & Marttinen (2006), we used 100 realizations from the posterior of the allele frequencies to determine admixture. We conducted the admixture analysis five times and the results were highly consistent among replicates. The full search algorithm of CLUMPP (Jakobsson & Rosenberg 2007) was used to obtain an optimal alignment from these replicates. This analysis did not include information on spatial locations of the samples, although we did implement such an analysis using both BAPS with spatial data (Corander *et al.* 2007) and TESS (Chen *et al.* 2007). These analyses are not presented, as the former produced results similar to those of STRUCTURE, while the latter produced similar results to those obtained with BAPS without spatial data.

Finally, we used a contingency test (Waples & Gaggiotti 2006) to define populations. This method is based on the analyses of contingency tables of allele frequency differences among sample sites. We implemented this approach using GENEPOP and the statistical criteria suggested by Waples & Gaggiotti (2006). In simulations, this ad hoc method was much more powerful than assignment tests, such as STRUCTURE, at identifying the underlying population structure when F_{ST} values were small. Additionally, Waples & Gaggiotti (2006) showed that this method rarely identifies population structure when it does not exist.

Nonmetric multidimensional scaling (NMS) was used to create a plot depicting the genetic relationships among sample sites in the program PC-ORD using the Euclidean distance measure (McCune & Mefford 1999). NMS ordination is a nonparametric projection in space, based in this case on allele frequencies, which reduced variation from six dimensions down to two dimensions in the final solution. The lack of correspondence between the original genetic distances and the ordination in two dimensions is called stress, with a value of 0% representing perfect correspondence.

Landscape composition

We quantified habitat between pairs of sample sites using the 2001 National Land-Cover Database (NLCD) (Homer *et al.* 2004), with a spatial resolution of 30 m, and FRAGSTATS (McGarigal *et al.* 2002). Because it was unclear how much of the area between sites should be considered when assessing habitat features, indices for habitat between pairs

of sites were calculated for both a 10 and 20 km wide band around the centreline extending from the centre points of each site. We used the 10 km assessments for our analyses because band-width had no effect on outcomes.

Because the golden-cheeked warbler is dependent on forest, we estimated the percent forest, percent agriculture, percent developed lands and the connectivity of forest patches between sites. The forest designation corresponds to the deciduous, evergreen and mixed forest categories. The developed designation corresponds to the developed open space and developed low, medium and high intensity categories. The agriculture designation corresponds to the cultivated crops, pasture/hay and grassland/herbaceous categories. All data were in the Universal Transverse Mercator coordinate system, a specialized Transverse Mercator projection, using North American Datum 1983 (NAD83).

Connectivity was calculated in FRAGSTATS using the class-level Connectance Index (McGarigal & Marks 1995). The Connectance Index is defined as the proportion of connected forest patches, where each pair of patches is either connected or not based on a Euclidian threshold distance, relative to the maximum possible connectance among all patches. We tested threshold distances of $1, 3.5$ and 9 km, based on the average breeding and natal dispersals of female and male golden-cheeked warblers (Ladd & Gass 1999); because choice of threshold did not affect outcomes, we used the intermediate value. Because connectivity was calculated as a class-level metric, a single value resulted for each intersite corridor. We used this measure of connectivity because we are interested in the long-term viability of the species as a result of habitat loss and fragmentation, and 'connectivity' is defined as 'the degree to which a landscape facilitates or impedes ecological flows' (McGarigal & Marks 1995). The Connectance Index calculates connectivity between individual patches, which is more appropriate for the scale of our analysis and is widely used in landscape ecology. Connectivity was estimated using both unfiltered (including all forest patches) and filtered (removing all forest patches < 250 ha that were > 1 km from forest patches > 250 ha) landcover data. These filtering criteria were used by Diamond & True (1998) to identify core golden-cheeked warbler breeding habitat areas. Because estimates of connectivity from the filtered and unfiltered landcover data were highly correlated ($r = 0.910, P < 0.001$), only results using the filtered data are presented.

Associations of geographical distance and connectivity, as well as percent forest, agriculture and developed land with genetic differences between populations, were assessed using Mantel tests (XLSTAT™). We evaluated these associations with two measures of genetic distance, F_{ST} and chord distance (Cavalli-Sforza & Edwards 1967). Chord distance was chosen because it tends to perform well with

Table 1 Sample size, location, estimated number of singing males and summary of genetic diversity for seven golden-cheeked warbler sample sites. Measures of genetic diversity include 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 nine polymorphic loci

Sample Site	Sample Size*	UTM Coordinates†		Singing Males	A	A_R	H_E	H_O	F_{IS}
		E	N						
KLR	14	640 899	3 559 975	35‡	7.11	7.11	0.74	0.77	-0.017
FHW	14	612 968	3 467 215	560§	7.78	7.78	0.75	0.75	0.038
FHE	14	635 032	3 455 678	4290§	7.78	7.78	0.76	0.71	0.093
FHS	19	607 587	3 438 313	2290§	8.78	8.12	0.76	0.75	0.039
BAC	17	597 729	3 387 421	1000¶	8.56	7.97	0.76	0.76	0.032
CAB	17	541 051	3 284 183	485**	7.78	7.30	0.74	0.76	0.000
KWM	14	451 473	3 327 018	26¶	7.78	7.78	0.75	0.78	-0.006
Mean					7.94	7.69	0.75	0.75	0.026

*All captured birds were males except for one female each from FHE, BAC and CAB; two females from KLR and three females from KWM, †North American Datum 1983 (NAD83), ‡(Glenda Miller, personal communication), §Calculated using available habitat (Scott Tweddle, personal communication) and average territorial male density (Jette *et al.* 1998), ¶USFWS (2004), **Cooksey & Thompson (2005).

microsatellite data and may produce a different suite of relationships among populations than F_{ST} , when most of the differences among populations might be due to recent genetic drift (Takezaki & Nei 1996; Kalinowski 2002). Cavalli-Edwards chord distance was estimated using PHYLIP (Felsenstein 1989). Estimates of differentiation based upon F_{ST} and chord distance were strongly correlated ($r = 0.820$, $P < 0.001$); however, choice of distance measure did have some effect on the outcome of tests of association between genetic differentiation and landscape features, so we present the results for both. Significance of correlation coefficients were evaluated using 100 000 permutations of the matrices. We predicted that genetic differences between populations would increase with increasing geographical distance, proportions of agricultural lands and proportions of developed lands; conversely, we expected genetic differences to decrease with increasing proportions of forest and increasing connectivity between sites. To control for intermatrix correlations, we used a partial Mantel test to assess the association of one measure of habitat or distance between sites with genetic differentiation, while holding the influence of a second measure 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).

Results

Genetic diversity

Over the 2004 and 2005 breeding seasons, we sampled a total of 109 individuals from seven sites (Table 1). None of the tests for deviation from HWE or for linkage disequilibrium were significant when the false discovery

rate was adjusted for multiple comparisons (Benjamini & Hochberg 1995).

The lowest allelic richness was found in KLR (Table 1); however, there was no variation in allelic richness ($F_{6,48} = 1.031$, $P = 0.417$) or heterozygosity ($F_{6,48} = 0.003$, $P = 1.000$) among sites. No sample site had significant heterozygote excess under mutation–drift equilibrium (Cornuet & Luikart 1996). None of the M ratios for individual sites were smaller than M_G and the M ratio for the total population sample was 0.72 (Garza & Williamson 2001). These two lines of evidence suggest that none of the samples from our study sites were from populations that have experienced a recent bottleneck.

Population structure

Overall, genetic differentiation among sample sites was relatively small, but highly significant ($F_{ST} = 0.008$, $P < 0.001$). The largest pairwise F_{ST} values were observed between KLR and all other sample sites, as well as between FHE and both CAB and KWM (Table 2).

The mixture analysis in BAPS consistently identified three clusters of individuals that were larger than the minimum threshold of three individuals per cluster suggested by Corander & Marttinen (2006). While most individuals fell into one large cluster, one genetically divergent group consisted of five individuals from KLR and a second group consisted of six individuals from FHW and FHE. The individuals identified as being genetically distinct in the mixture analysis were assigned to the same divergent groups in the admixture analysis (Fig. 2). Outside of KLR and the combined sample of FHW and FHE, there was little variation in the level of admixture among samples. The method of Waples &

Table 2 Pairwise estimates of F_{ST} (lower left) and chord distance (upper right) from tests of null hypothesis of no genetic differentiation among seven golden-cheeked warbler sample sites. Overall $F_{ST} = 0.008$ ($P < 0.001$)

	KLR	FHW	FHE	FHS	BAC	CAB	KWM
KLR		0.059	0.066	0.067	0.068	0.049	0.059
FHW	0.016*		0.037	0.035	0.042	0.038	0.042
FHE	0.013*	0.000		0.056	0.052	0.056	0.060
FHS	0.026*	0.000	0.003		0.045	0.037	0.045
BAC	0.036*	0.000	0.009	0.006		0.039	0.037
CAB	0.016*	0.005	0.019*	0.002	0.003		0.035
KWM	0.024*	0.001	0.008*	0.000	0.000	0.002	

*Significant P -values ($P < 0.05$) following adjustment for multiple comparisons (Benjamini & Hochberg 1995).

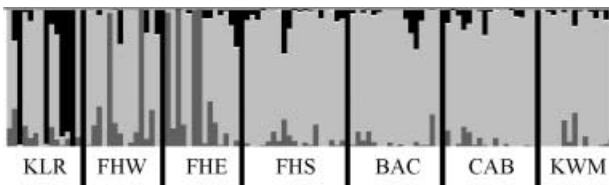


Fig. 2 Admixture coefficients for golden-cheeked warblers estimated using bAPS. Each vertical column corresponds to one individual. Columns are shaded with different colours (black, dark grey and light grey) in proportions corresponding to estimated admixture coefficients for each individual. Individuals from the different sample sites are separated by black vertical lines.

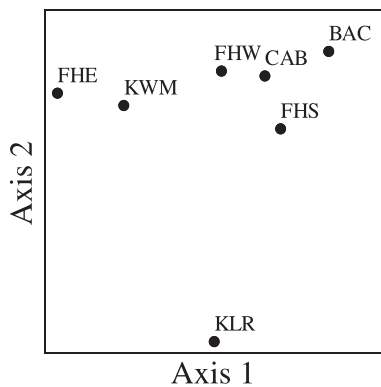


Fig. 3 Nonmetric multidimensional scaling (NMS) ordination two-dimensional plot representing the relative genetic relationships among golden-cheeked warblers from seven sample sites.

Gaggiotti (2006) identified KLR as being differentiated from all other sites. No other individual or set of sample sites was sufficiently differentiated from others to be considered a separate population.

Reduction of the allele-frequency differences among sample sites down to two axes resulted in a stress of 3.7% (Fig. 3). Kruskal (1964) notes that NMS ordinations with a stress of $< 10\%$ represent excellent depictions of the

relationships among the original data. When interpreting the NMS plot it is important to note that, while this approach resulted in less distortion of relationships among sites than other ordination approaches because it does not assume linear relationships among allele frequencies, the numbering of the dimensions had no correlation to the amount of variation explained by each dimension, and it was not possible to directly attribute the effects of individual loci to individual dimensions. In this two-dimensional space, KLR appeared to be the most different from other sites, reflecting the relatively high pairwise F_{ST} values for this site and the other sites examined (Fig. 3). Although FHE was also differentiated from several sites, it does not occupy as unique an area of the ordination of genetic space as KLR (Fig. 3).

Landscape composition

There were associations of connectivity ($r = -0.40$, $P = 0.011$) and geographic distance ($r = 0.40$, $P = 0.037$) with F_{ST} . As expected, the genetic distances between sites increased as the connectivity of sites decreased (Fig. 4a) and as geographic distance between sites increased (Fig. 4c). Controlling for covariation with geographic distance, the association between connectivity and F_{ST} was no longer significant ($r = -0.14$, $P = 0.278$). Likewise, there was no association between geographic distance and F_{ST} when controlling for covariation with connectivity ($r = 0.16$, $P = 0.248$). The difficulty of determining the relative contributions of geographic distance and connectivity to F_{ST} is due to the strong relationship between connectivity and geographic distance ($r = -0.80$, $P < 0.001$). In contrast to F_{ST} , chord distance was not associated with connectivity ($r = -0.28$, $P = 0.115$) or geographic distance ($r = 0.22$, $P = 0.167$; Fig. 4d).

Unlike connectivity, geographic distance was not strongly correlated to the percentage of forested land ($r = -0.04$, $P = 0.368$) or agriculture ($r = -0.23$, $P = 0.306$) between sites, although there was a negative correlation between the percentage of developed land and geographic distance between sites ($r = -0.43$, $P = 0.018$). There was no significant negative association between F_{ST} and the percentage of forested land ($r = -0.29$, $P = 0.101$). Contrasting F_{ST} , chord distance was strongly correlated with the percentage of forested land ($r = -0.50$, $P = 0.009$; Fig. 4b), and these relationships were not altered by holding geographic distance constant. Likewise, there was no positive association between either F_{ST} or chord distance with the percentage of developed land, regardless of whether geographic distance was held constant ($P > 0.100$). As predicted, there was a strong positive association between F_{ST} and the percentage of agricultural land between sample sites ($r = 0.55$, $P = 0.008$; Fig. 4e). This association between F_{ST} and the percentage of agricultural land increased when the

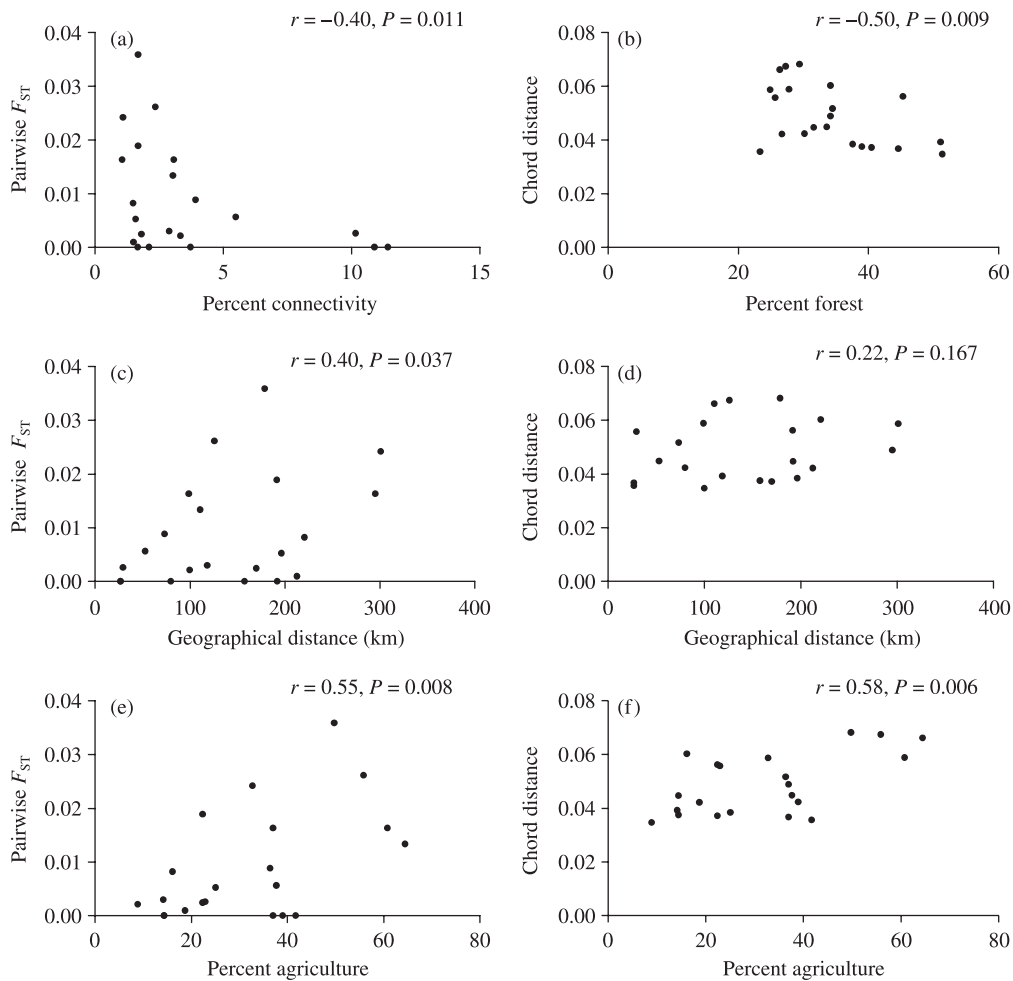


Fig. 4 Associations of pairwise estimates of F_{ST} and (a) percent connectivity, (c) geographical distance and (e) percent agriculture; and of pairwise estimates of chord distance and (b) percent forest, (d) geographical distance and (f) percent agriculture among golden-cheeked warbler samples from seven sites.

influence of geographic distance was removed from the analysis ($r = 0.72$, $P < 0.001$). A similar relationship was observed between chord distance and the percentage of agricultural land ($r = 0.58$, $P = 0.006$; Fig. 4f), and again the association increased when geographic distance was held constant ($r = 0.67$, $P < 0.001$). All P -values < 0.03 in these analyses remained significant when adjusted for false detections.

Discussion

Genetic diversity

Although the golden-cheeked warbler has suffered significant habitat loss and fragmentation over the past few decades, estimates of allelic richness and heterozygosity are relatively high and similar to those of other warbler species (Gibbs *et al.* 2000; Clegg *et al.* 2003; Veit *et al.* 2005).

Even with an estimated 25% reduction in the number of breeding pairs between 1962 and 1990 (USFWS 1992), the overall population size of 5900–7000 singing males (USFWS 2004) has thus far been sufficiently large to retain high levels of genetic diversity.

There were no differences in allelic richness or heterozygosity among sampled sites, suggesting no difference in the bottleneck histories of the individual sites. Allelic richness is known to be especially sensitive to small population sizes (Leberg 1992; Spencer *et al.* 2000). There was also no evidence of heterozygote excess or a shift in the distribution of allele sizes typical of populations experiencing bottlenecks (Cornuet & Luikart 1996; Garza & Williamson 2001). The small numbers of singing males in some sample sites (Table 1), likely reflecting even smaller numbers of breeding pairs, could result in the genetic signature of bottlenecks were those sites isolated. The lack of any genetic evidence of a bottleneck suggests the importance

of gene flow for maintaining genetic diversity across populations of this species (Slatkin 1985).

In addition to the potential role of gene flow in minimizing the signature of bottlenecks, it is possible that bottlenecks were not detected due to a lack of statistical power. For the number of loci in our study, both approaches used to test for recent bottlenecks in our sample sites lack power to detect reductions in populations when the minimum $N_e > 50$ (Luikart & Cornuet 1998; Garza & Williamson 2001; Leblois *et al.* 2006). It is possible that populations experienced bottlenecks, but that those bottlenecks were not severe enough to be detected using nine loci.

Population structure

Current levels of gene flow among sample sites were limited enough to have resulted in the significant differentiation of at least one population. The amount of genetic structure observed was strong enough to assign individuals to three different groups using BAPS and two distinct groups using the contingency table method. In both approaches, as well as in the NMS ordination, KLR was genetically differentiated from other sites.

The observed differentiation among sample sites, with an overall F_{ST} value of 0.008, was slightly smaller than estimates of population structure for other warbler species (0.010–0.035; Gibbs *et al.* 2000; Clegg *et al.* 2003; Veit *et al.* 2005). The estimates of F_{ST} for these species, however, were made over breeding ranges that were 10–200 times the size of the breeding range of the golden-cheeked warbler. When considering only the geographically closest populations in other warbler studies (similar to the maximum distance between our sampled sites), pairwise F_{ST} values were between 0.006 and 0.015 for *Dendroica petechia* (Gibbs *et al.* 2000), 0.002 and 0.006 for *Wilsonia pusilla* (Clegg *et al.* 2003) and –0.013 and 0.039 for *Dendroica cerulea* (Veit *et al.* 2005). Pairs of our sites separated by comparable distances (~300 km) had F_{ST} values between 0.016 and 0.024, indicating that genetic structure in the golden-cheeked warbler is at the high end of that observed for other warbler species on a similar spatial scale.

Avian species can be expected to exhibit low levels of population differentiation due to high rates of dispersal (Crochet 2000). Observed dispersal patterns of golden-cheeked warblers seem to be fairly typical of migratory passerines, with natal dispersal being more extensive than breeding dispersal (Villard *et al.* 1995). Although adult breeders have been observed to disperse < 1 km on average from their location in a previous year (Ladd & Gass 1999), average dispersal from the natal site is 9 km for males and 3 km for females (Ladd & Gass 1999). Because these observations are a function of survey efforts at specific sites (non-random sampling), they may underestimate actual mean dispersal distances.

Landscape composition

Although the potential for dispersal was high, there were strong differences in allele frequencies among some sites and evidence that the type of habitat features between sites was influencing this genetic differentiation. Analysis of F_{ST} supported our prediction that genetic differentiation would increase as connectivity decreased; however, our analysis of chord distance did not. The geographic distance between sites also had a significant association with F_{ST} . Because connectivity and geographic distance were highly correlated, it is not possible to distinguish between the influence of isolation-by-distance and fragmentation on genetic differentiation in this dataset. Forest cover had a strong, positive association with chord distance, but not with F_{ST} . The different associations of our two measures of genetic differentiation with connectivity, geographic distance and percent forest cover lead to the question of which measure of genetic differentiation would be more reliable in this case. One could argue that because chord distance does not make the assumption that populations are in drift-mutation equilibrium, it might be a better choice of measure of genetic differentiation than F_{ST} in recently fragmented populations. This assumption, however, applies only to the use of F_{ST} as an estimator of gene flow, and nonequilibrium conditions should not affect its use as an index of relative genetic differentiation (Neigel 2002). It is not clear whether more weight should be placed on one measure or the other, as each responds to drift, mutation and gene flow in different ways (Kalinowski 2002).

The only habitat feature that had a strong association with both measures of genetic differentiation was the percentage of agricultural land. A decreased likelihood of birds to cross agricultural landscapes would explain why the greatest observed differentiation was between the northernmost site (KLR) and the other sites, as KLR was largely surrounded by agricultural lands. It is important to recognize that our measures of connectivity and forest cover are not measurements of the distribution of preferred nesting habitat, as there is only limited spatial data on the locations of dense, mature stands of Ashe juniper and oaks. While fine-scale characterization of habitat preference in dispersing golden-cheeked warblers is currently lacking, it may be that like many woodland passerines, the presence of general forest cover may play a greater role in dispersal than the actual composition of the forest (Wiens 1994; Harris & Reed 2002). It is possible that the negative associations of both measures of genetic differentiation with agricultural lands was stronger than their positive associations with connectivity or forest cover because all agricultural lands are essentially uninhabitable to the golden-cheeked warbler, while only a portion of the forested habitat may

be suitable for the species. The significant associations between genetic distance and habitat were strongly influenced by KLR. It is the most differentiated site and also the most isolated from the other sites in terms of intervening agricultural habitat. When KLR was removed from the analyses, associations between genetic differentiation and habitat variables were no longer significant, while the removal of other sites did not have a large effect on the associations.

Why would a bird capable of migrating thousands of kilometres exhibit reduced genetic exchange between KLR and large populations just 70 km to the south? The species is relatively philopatric, with the vast majority of individuals nesting within 4 km of where they were born (Ladd & Gass 1999). This pattern might result in isolation-by-distance, but does not explain why the KLR and FHE sites, which are separated by agricultural land, were much more differentiated than other pairs of sites separated by similar distances, but with larger tracts of intervening forest. One possibility is that low dispersal may result from the tendency of forest-dwelling birds to avoid crossing open areas (Dickman 1987; Belisle *et al.* 2001). These effects are most restrictive for species with specialized habitat requirements (Desrochers & Hannon 1997; Harris & Reed 2002), such as golden-cheeked warblers. Although many birds migrate large distances, most tend to breed near their natal site (Gauthreaux 1982; Greenwood & Harvey 1982), and dispersal movements to locate new breeding territories likely depend on local landscape features (Opdam 1991; Belisle *et al.* 2001). Long-distance dispersal by forest-dependent birds across an agricultural landscape would be much less likely than dispersal across a landscape dominated by forest and shrubland, leading to differentiation of populations isolated by agricultural land. It is also possible that the size of a habitat fragment might play a role in the ability of dispersers to locate that patch (Williams 1964; Freemark & Merriam 1986). The KLR site is relatively small and is located at the northern end of the current breeding distribution of the species, making it possible that the few birds dispersing in that direction might not encounter the small habitat patches supporting the population. Available data do not allow us to differentiate between these hypotheses.

Although declines in golden-cheeked warbler population size during the last 50 years were substantial enough to result in the species being listed as endangered (USFWS 1992), effective population sizes have not yet become small enough to result in serious erosion of genetic diversity. There is, however, the possibility that insufficient time since fragmentation has occurred to detect bottleneck effects in those populations with very low numbers of singing males. Because brown-headed cowbird control has apparently resulted in stabilizing or increasing populations in some

areas (Hayden *et al.* 2000), there is reason to be optimistic about the future of the species. If current trends of habitat alteration continue in central Texas, however, connectivity may further erode, reducing gene flow that has probably thus far mitigated the effects of small population sizes on genetic variation.

The recovery plan for the species established eight recovery units within the current breeding range (Fig. 1) and called for the maintenance of at least one viable population in each recovery unit (USFWS 1992). We could not obtain samples from three of the units (Fig. 1) because known sites contained only a few singing males or managers deemed that sampling efforts might disrupt population recovery. This may have affected our results, possibly underestimating the effects of fragmentation. Additionally, our capture methods resulted in a male-biased sample. Because female golden-cheeked warblers tend to disperse shorter distances than males, this bias may also contribute to underestimated fragmentation effects. Analysis of samples obtained from the other five recovery units indicated no evidence of population divergence suggestive of long-term genetic isolation. The species does not appear to contain any evolutionary significant units (Waples 1991) and there is no compelling genetic basis for managing populations as separate entities, although such a strategy has other merits such as minimizing risk to the species due to unexpected declines in one or a few populations.

The two northernmost units (Fig. 1) are currently known to contain only eight sites, with between five and 16 singing males per site (USFWS 2004), in addition to the small KLR population we sampled. Because the number of breeding pairs is small, drift will eventually increase levels of genetic differentiation with these northern populations. This differentiation could become exacerbated if population sizes or immigration rates are further reduced. Increased isolation would also put these small populations at a greater risk of local extinction. Because recent trends have been towards further reductions in connectivity among habitat patches in this region, protecting existing patches and fostering late-successional growth at other sites could improve connectivity, potentially improving the interpopulation genetic dynamics of these northern populations and safeguarding the long-term genetic viability of the species.

Conclusions

Few migratory birds have exhibited genetic structure on their breeding grounds at the spatial scale of the golden-cheeked warbler. We conclude that a lack of connectivity among habitat patches, resulting from loss of natural landscapes, is likely playing a primary role in the notable genetic differentiation of at least one population (KLR) and is of concern for other populations as well. The influence

of habitat fragmentation on genetic differentiation has important implications for management of species like the golden-cheeked warbler that are relatively vagile, but highly specialized in their habitat preferences. Restoration and protection of connected patches is likely to be the best option for conserving or recovering such species (Young & Clarke 2000).

Landscape genetic approaches, applied to potentially vagile groups such as the butterfly *Parnassius smintheus*, have demonstrated that connectivity influences rates and patterns of gene flow and thus the amount and distribution of genetic diversity (Keyghobadi *et al.* 2005). Studies of nonmigratory avian species have also suggested that habitat fragmentation can affect genetic structure (Bates 2002; Segelbacher *et al.* 2003). Although both traditional and landscape approaches have suggested habitat fragmentation can contribute to genetic structure, the influence of landscape composition on highly vagile organisms, such as migratory birds, is unclear. Our results suggest that in spite of the ability to transverse large expanses of inhospitable habitat during migration, fragmentation of breeding habitat has the potential to result in genetic differentiation.

The behavioural limitations of birds to disperse across fragmented landscapes have been predicted to increase with increasing habitat specialization (Harris & Reed 2002). The only other study that we are aware of that addresses the genetic implications of fragmentation for a migratory passerine, the cerulean warbler (*Dendroica cerulea*), failed to observe an association between fragmentation and genetic structure (Veit *et al.* 2005), although the species has less restrictive breeding habitat requirements than the golden-cheeked warbler. It is possible that our observation of increased genetic differentiation for a population isolated by land-use conversion is due to the specialized habitat requirements of our study species, however, considerably more research on both habitat generalists and specialists is needed to evaluate the degree to which behavioural limitations on dispersal associated with habitat specialization influence genetic structure.

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References

- Bates JM (2000) Allozymic genetic structure and natural habitat fragmentation: data for five species of Amazonian forest birds. *Condor*, **102**, 770–783.
- Bates JM (2002) The genetic effects of forest fragmentation on five species of Amazonian birds. *Journal of Avian Biology*, **33**, 276–294.
- Belisle M, Desrochers A, Fortin MJ (2001) Influence of forest cover on the movements of forest birds: a homing experiment. *Ecology*, **82**, 1893–1904.
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, **57**, 289–300.
- Bouzat JL (2001) The population genetic structure of the Greater Rhea (*Rhea americana*) in an agricultural landscape. *Biological Conservation*, **99**, 277–284.
- Brown LM, Ramey RR, Tamburini B, Gavin TA (2004) Population structure and mitochondrial DNA variation in sedentary Neotropical birds isolated by forest fragmentation. *Conservation Genetics*, **5**, 743–757.
- Caizergues A, Ratti O, Helle P *et al.* (2003) Population genetic structure of male Black Grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. *Molecular Ecology*, **12**, 2297–2305.
- Castellano S, Balletto E (2002) Is the partial mantel test inadequate? *Evolution*, **56**, 1871–1873.
- Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics*, **19**, 233–257.
- Chen CE, Forbes DF, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, **7**, 747–756.
- Chuang-Dobbs HC, Webster MS, Holmes RT (2001) Paternity and parental care in the black-throated blue warbler, *Dendroica caerulescens*. *Animal Behaviour*, **62**, 83–92.
- 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.
- Cooksey ML, Thompson JE (2005) Monitoring the golden-cheeked warbler and the black-capped vireo on Camp Bullis. *Texas: 2005 Field Season Report*. Fort Sam Houston, Texas.
- Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, **15**, 2833–2843.
- Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367–374.
- Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, **20**, 2363–2369.
- Corander J, Marttinen P, Mantyniemi S (2006) Bayesian identification of stock mixtures from molecular marker data. *Fisheries Bulletin*, **104**, 550–558.
- Corander J, Sirén J, Arjas E (2007) Bayesian spatial modeling of genetic population structure. *Computational Statistics*, **23**, 111–129.
- 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.
- Crochet PA (2000) Genetic structure of avian populations — allozymes revisited. *Molecular Ecology*, **9**, 1463–1469.

- Dawson RJG, Gibbs HL, Hobson KA, Yezerinac SM (1997) Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warbler), and their use in population studies. *Heredity*, **79**, 506–514.
- Desrochers A, Hannon SJ (1997) Gap crossing decisions by forest songbirds during the post-fledging period. *Conservation Biology*, **11**, 1204–1210.
- Diamond DD, True CD (1998) Golden-cheeked warbler habitat area, habitat distribution, and change and brief analysis of land cover within the Edwards Aquifer recharge zone. *Final Report, Submitted to the Office of Endangered Species*, U. S. Fish and Wildlife Service, Albuquerque, New Mexico.
- Dickman CR (1987) Habitat fragmentation and vertebrate species richness in an urban environment. *Journal of Applied Ecology*, **24**, 337–351.
- Ellegren H, Carlson A, Stenberg I (1999) Genetic structure and variability of White-backed Woodpecker (*Dendrocopos leucotos*) populations in northern Europe. *Heredity*, **130**, 291–299.
- Felsenstein J (1989) PHYLIP – Phylogeny inference package Version 3.2. *Cladistics*, **5**, 164–166.
- Fish U.S. Wildlife Service (1992) *Golden-Cheeked Warbler (Dendroica Chrysoparia) Recovery Plan*. Albuquerque, New Mexico.
- Frankham R (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305–327.
- Freemark KE, Merriam HG (1986) Importance of area and habitat heterogeneity to bird assemblages in temperate forest fragments. *Biological Conservation*, **36**, 115–141.
- Galbusera P, Githiru M, Lens L, Matthysen E (2004) Genetic equilibrium despite habitat fragmentation in an Afrotropical bird. *Molecular Ecology*, **13**, 1409–1421.
- Garza JC, Williamson E (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.
- Gauthreaux SA (1982) The ecology and evolution of avian migration systems. In: *Avian Biology* (eds Farner DS, King JR), pp. 93–167. Academic Press, New York.
- 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.
- Goudet J (2001) FSTAT Version 2.9.3. a program to estimate and test gene diversities and fixation indices. <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Greenwood PJ, Harvey PH (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics*, **13**, 1–21.
- Harris RJ, Reed JM (2002) Behavioral barriers to non-migratory movements of birds. *Annales Zoologici Fennici*, **39**, 275–290.
- Hayden TJ, Tazik DJ, Melton RH, Cornelius JD (2000) Cowbird control program on Fort Hood, Texas: lessons for mitigation of cowbird parasitism on a landscape scale. In: *The Ecology and Management of Cowbirds* (eds Cook T, Robinson S, Rothstein S, Sealy S, Smith J), pp. 357–370. The University of Texas Press, Austin, TX.
- 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.
- 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.
- Jette LA, Hayden TJ, Cornelius JD (1998) Demographics of the golden-cheeked warbler (*Dendroica Chrysoparia*) on Fort Hood, Texas. Construction Engineering Research Laboratory, US Army Engineer Research and Development Center, Champaign, Illinois. *Technical Report 98/52*.
- Johnson DH (2001) Habitat fragmentation effects on birds in grasslands and wetlands: a critique of our knowledge. *Great Plains Research*, **11**, 211–213.
- Kalinowski ST (2002) Evolutionary and statistical properties of three genetic distances. *Molecular Ecology*, **11**, 1263–1273.
- Keyghobadi N, Roland J, Strobeck C (2005) Genetic differentiation and gene flow among populations of the alpine butterfly, *Parnassius smintheus*, vary with landscape connectivity. *Molecular Ecology*, **14**, 1897–1909.
- Kruskal JB (1964) Nonmetric multidimensional scaling: a numerical method. *Psychometrika*, **29**, 115–130.
- Ladd C, Gass L (1999) Golden-cheeked warbler: *Dendroica chrysoparia*. In: *The Birds of North America*, (eds Poole A, Gill F), 420, pp. 1–23. Cornell Laboratory of Ornithology and the Academy of Natural Sciences, Ithaca, New York.
- Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE Jr (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295–302.
- Leberg PL (1991) Influence of fragmentation and bottlenecks on genetic-divergence of wild turkey populations. *Conservation Biology*, **5**, 522–530.
- Leberg PL (1992) Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution*, **46**, 477–494.
- Leblois R, Estoup A, Streiff R (2006) Genetics of recent habitat contraction and reduction in population size: does isolation by distance matter? *Molecular Ecology*, **15**, 3601–3615.
- Luikart GL, Cornuet JM (1998) Empirical evaluation of a test for detecting recent historical population bottlenecks. *Conservation Biology*, **12**, 228–237.
- McCune B, Mefford MJ (1999) *PC-ORD, Multivariate Analysis of Ecological Data, Version 4*. MjM Software Design, Gleneden Beach, OR.
- McGarigal K, Marks BJ (1995) *FRAGSTATS: Spatial Pattern Analysis Program for Quantifying Landscape Structure*. USDA, Portland, Oregon.
- McGarigal K, Cushman SA, Neel MC, Ene E (2002) *FRAGSTATS: Spatial Pattern Analysis Program for Categorical Maps*. University of Massachusetts, Amherst, MS. <http://www.umass.edu/landeco/research/fragstast/fragstats.html>.
- Neigel JE (2002) Is F_{ST} obsolete? *Conservation Genetics*, **3**, 167–173.
- Opdam P (1991) Metapopulation theory and habitat fragmentation: a review of holarctic breeding bird studies. *Landscape Ecology*, **5**, 93–106.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Rappole JH, King DI, Diez J (2003) Winter vs. breeding habitat limitation for an endangered avian migrant. *Ecological Applications*, **13**, 735–742.
- 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 ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Rousset F (2002) Partial mantel tests: reply to Castellano and Balletto. *Evolution*, **56**, 1874–1875.

- SAS Institute (2005) *SAS Onlinedoc 9.1.3*. SAS Institute Inc, Cary, NC.
- Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology*, **5**, 18–32.
- Segelbacher G, Höglund J, Storch I (2003) From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. *Molecular Ecology*, **12**, 1773–1780.
- Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.
- 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 CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology*, **9**, 1517–1528.
- Storfer A, Murphy MA, Evans JS *et al.* (2007) Putting the 'landscape' in landscape genetics. *Heredity*, **98**, 128–142.
- Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, **144**, 389–399.
- U.S. Fish and Wildlife Service (2004) Biological Opinion, Consultation Number 2–12-05-F-021.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley PF (2003) *Micro-Checker*. The University of Hull, Hull.
- Veit ML, Robertson RJ, Hamel PB, Friesen VL (2005) Population genetic structure and dispersal across a fragmented landscape in Cerulean warblers (*Dendroica cerulea*). *Conservation Genetics*, **6**, 159–174.
- Villard M-A, Merriam G, Maurer BA (1995) Dynamics in subdivided populations of neotropical migratory birds in a fragmented temperate forest. *Ecology*, **76**, 27–40.
- Villard M-A, Trzcinski MK, Merriam G (1999) Fragmentation effects on forest birds: relative influence of woodland cover and configuration on landscape occupancy. *Conservation Biology*, **13**, 774–783.
- Wahl R, Diamond DD, Shaw D (1990) The golden-cheeked warbler: a status review. *Final report, submitted to the Office of Endangered Species*, U. S. Fish and Wildlife Service, Albuquerque, New Mexico.
- Waples RS (1991) Genetic interactions between hatchery and wild salmonids — Lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 124–133.
- 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.
- Webster MS, Chuang-Dobbs HC, Holmes RT (2001) Microsatellite identification of extrapair sires in a socially monogamous warbler. *Behavioral Ecology*, **12**, 439–446.
- Wiens JA (1994) Habitat fragmentation: Island v landscape perspectives on bird conservation. *Ibis*, **137**, S97–S104.
- Williams CB (1964) *Patterns in the Balance of Nature and Related Problems in Quantitative Ecology*. Academic Press, London.
- Winker K, Glenn TC, Graves GR (1999) Dinucleotide microsatellite loci in a migratory wood warbler (Parulidae: *Limnothlypis swainsonii*), and amplification among other songbirds. *Molecular Ecology*, **8**, 1553–1556.
- Yezerinac SM, Gibbs HL, Briskie JV, Whittam R, Montgomerie R (1999) Extrapair paternity in a far northern population of yellow warblers *Dendroica petechia*. *Journal of Avian Biology*, **30**, 234–237.
- Young AC, Clarke GM (2000) *Genetics, Demography and the Viability of Fragmented Populations*. Cambridge University Press, Cambridge.

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