

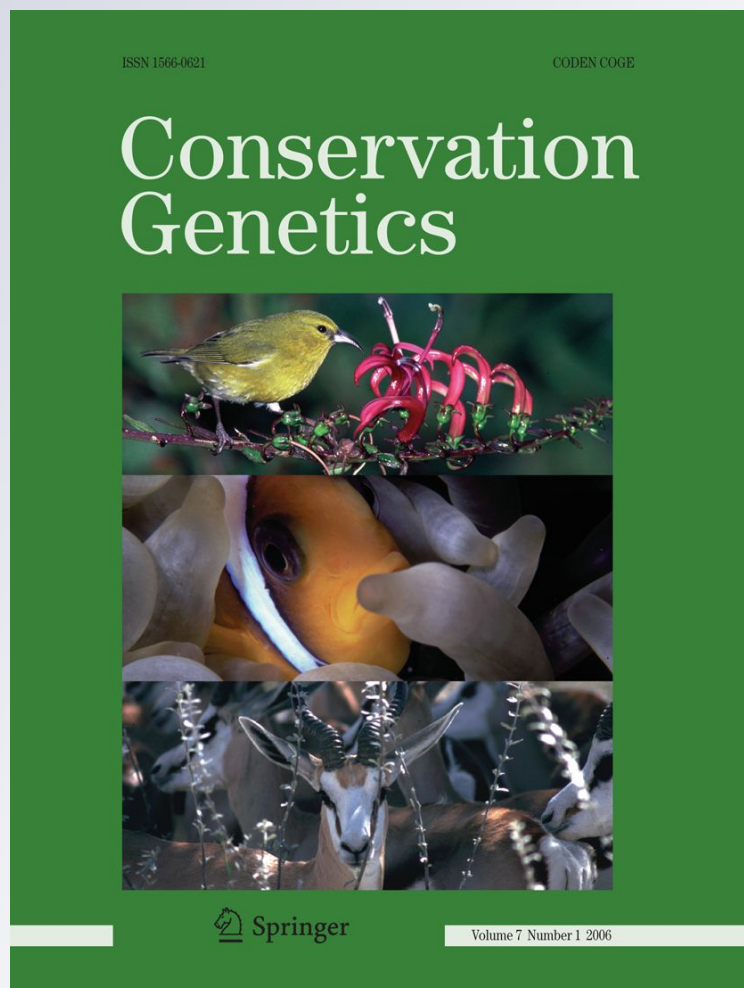
*Crumbling diversity: comparison of historical archived and contemporary natural populations indicate reduced genetic diversity and increasing genetic differentiation in the golden-cheeked*

*Warbler Athrey, Denise L. Lindsay, Richard F. Lance & Paul L. Leberg*

**Conservation Genetics**

ISSN 1566-0621

Conserv Genet  
DOI 10.1007/  
s10592-011-0235-8



 Springer

## Report Documentation Page

*Form Approved*  
*OMB No. 0704-0188*

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE <b>JUN 2011</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2011 to 00-00-2011</b>	
4. TITLE AND SUBTITLE <b>Crumbling Diversity: Comparison Of Historical Archived And Contemporary Natural Populations Indicate Reduced Genetic Diversity And Increasing Genetic Differentiation In The Golden-Cheeked Warbler</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>U. S. Army Engineer Research And Development Center, Vicksburg, MS, 39180</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES <b>Conservation Genetics, 2011</b>					
14. ABSTRACT <b>Genetic viability of threatened and endangered species is of increasing concern with habitat loss and fragmentation. Valuable assessments of the genetic status of endangered species are difficult in most cases, where only single sample estimates are available. Using historical and contemporary samples, we assessed the impact of both historical and recent demographic changes on population genetics of the endangered golden-cheeked warbler, (<i>Dendroica chrysoparia</i>). Our study documents a steep decline in genetic diversity in an endangered species over a 100-year period, along with concurrent increase in genetic differentiation, and low contemporary effective sizes for all the populations we evaluated. While adding to the growing body of literature that describes the genetic impacts of habitat fragmentation, our study may also serve as an informative guide to future management of endangered species. Our study underlines the importance of long term population genetic monitoring in understanding the full extent of genetic changes in endangered species.</b>					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

**Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media B.V.. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.**

# Crumbling diversity: comparison of historical archived and contemporary natural populations indicate reduced genetic diversity and increasing genetic differentiation in the golden-cheeked warbler

Giridhar Athrey · Denise L. Lindsay ·  
Richard F. Lance · Paul L. Leberg

Received: 29 December 2009 / Accepted: 2 June 2011  
© Springer Science+Business Media B.V. 2011

**Abstract** Genetic viability of threatened and endangered species is of increasing concern with habitat loss and fragmentation. Valuable assessments of the genetic status of endangered species are difficult in most cases, where only single sample estimates are available. Using historical and contemporary samples, we assessed the impact of both historical and recent demographic changes on population genetics of the endangered golden-cheeked warbler, (*Dendroica chrysoparia*). Our study documents a steep decline in genetic diversity in an endangered species over a 100-year period, along with concurrent increase in genetic differentiation, and low contemporary effective sizes for all the populations we evaluated. While adding to the growing body of literature that describes the genetic impacts of habitat fragmentation, our study may also serve as an informative guide to future management of endangered species. Our study underlines the importance of long term population genetic monitoring in understanding the full extent of genetic changes in endangered species.

**Keywords** Genetic diversity · Fragmentation · Effective population size · Endangered birds · Historical-contemporary samples

Habitat loss and fragmentation are among the greatest threats to biodiversity in the 21st century (Young and Clarke 2000). Species that are habitat specialists and require large home ranges are particularly sensitive to habitat loss and fragmentation and may experience increased genetic drift and reduced gene flow. While a number of studies have examined the role of fragmentation on genetic structure (Young et al. 1996; Martinez-Cruz et al. 2007; Howeth et al. 2008; Sato and Harada 2008; Yamamoto et al. 2004), these assessments are often restricted to contemporary patterns, when examination of temporal changes would provide considerably more information. Despite the burgeoning evidence for the implications of habitat fragmentation on contemporary populations (Rodriguez-Munoz et al. 2007; Segelbacher et al. 2008), it is difficult to tease out the magnitude or trajectory of demographic and genetic change accompanying fragmentation, so as to inform conservation management. This is usually compounded by the unavailability of pre-fragmentation or ancestral population samples as reference. Although typing specimens from museum archives is being embraced for various evolutionary and population genetic studies, its application has been relatively limited, owing to incomplete geographical representation or limited sampling. This is especially so for direct assessments of the effects of recent habitat fragmentation on contemporary population structure and genetic diversity. However, when applied, this approach has yielded valuable demographic or conservation relevant information in other species (Nichols et al. 2001).

**Electronic supplementary material** The online version of this article (doi:10.1007/s10592-011-0235-8) contains supplementary material, which is available to authorized users.

G. Athrey (✉) · P. L. Leberg  
Department of Biology, University of Louisiana,  
P.O. Box 42451, Lafayette, LA 70504, USA  
e-mail: giri.athrey@tamu.edu

D. L. Lindsay · R. F. Lance  
Environmental Laboratory, U.S. Army Engineer Research  
and Development Center, Vicksburg, MS 39180, USA

G. Athrey  
Vector Biology Group, Texas A&M University,  
2475 TAMU, College Station, TX 77843, USA

Identifying temporal changes in genetic variation may be especially valuable in the case of endangered species, as such data might indicate whether the effective population size has been small enough to result in reduced genetic variation (and thus adaptive potential) and if populations have become recently isolated and thus subject to local extirpation. Temporal changes in genetic variance are also valuable for understanding the effective size ( $N_e$ ) in relation to the census size ( $N_c$ ) because of the disparity between the number of individuals in a population and actual genetic contributors to each generation (Nei and Tajima 1981; Crow and Denniston 1988; Frankham 1995a). Another advantage of a temporal analysis is in facilitating quantification of genetic change in populations, partly satisfying the proximate data demands in a conservation context.

We present our results from temporal analyses of genetic data from the endangered golden-cheeked warbler (*Dendroica chrysoparia*) from samples spanning the years 1890–2005. In this study we were able to utilize archived museum samples as reference populations to understand the impacts of habitat fragmentation on contemporary populations of golden-cheeked warblers. This migratory songbird has a breeding range restricted to central Texas, USA (Fig. 1). Estimates of population sizes based on banding data and surveys were 18,500 and 13,800 in 1962 and 1990, respectively, with a 35% decline in breeding habitat (USFWS 1992). Preferred nesting habitat for this species are climax forests of Ashe juniper (*Juniperus ashei*) and oak (*Quercus* spp.) that develop on soils derived from limestone substrates (Kroll 1980; USFWS 1992). Similar to several other neotropical songbirds, golden-cheeked warblers exhibit site fidelity, territoriality and natal philopatry—all of which may have implications for population structure. Fragmentation of available habitat is known to have resulted from clearing of woodlands for livestock production and agriculture. Populations are also depressed due to brood parasitism by brown-headed cowbirds, *Molothrus ater* (Sperry et al. 2008).

In an earlier assessment of genetic structure in *D. chrysoparia* we found that few populations were significantly differentiated, with little evidence of genetic bottlenecks, and genetic diversity comparable to more abundant warbler species (Lindsay et al. 2008). As with similar studies, that investigation was based on contemporary samples alone. However, extrapolating from data based on a single time point limits the resolution of the full extent of recent and ongoing genetic changes. More complete assessments of genetic changes require assessing the temporal component of genetic change. Here we used samples taken from 2 to 3 time points spanning across a century, for three sites in central Texas (Fig. 1) to examine temporal change in genetic characteristics and estimate the effective population size ( $N_e$ ).

## Materials and methods

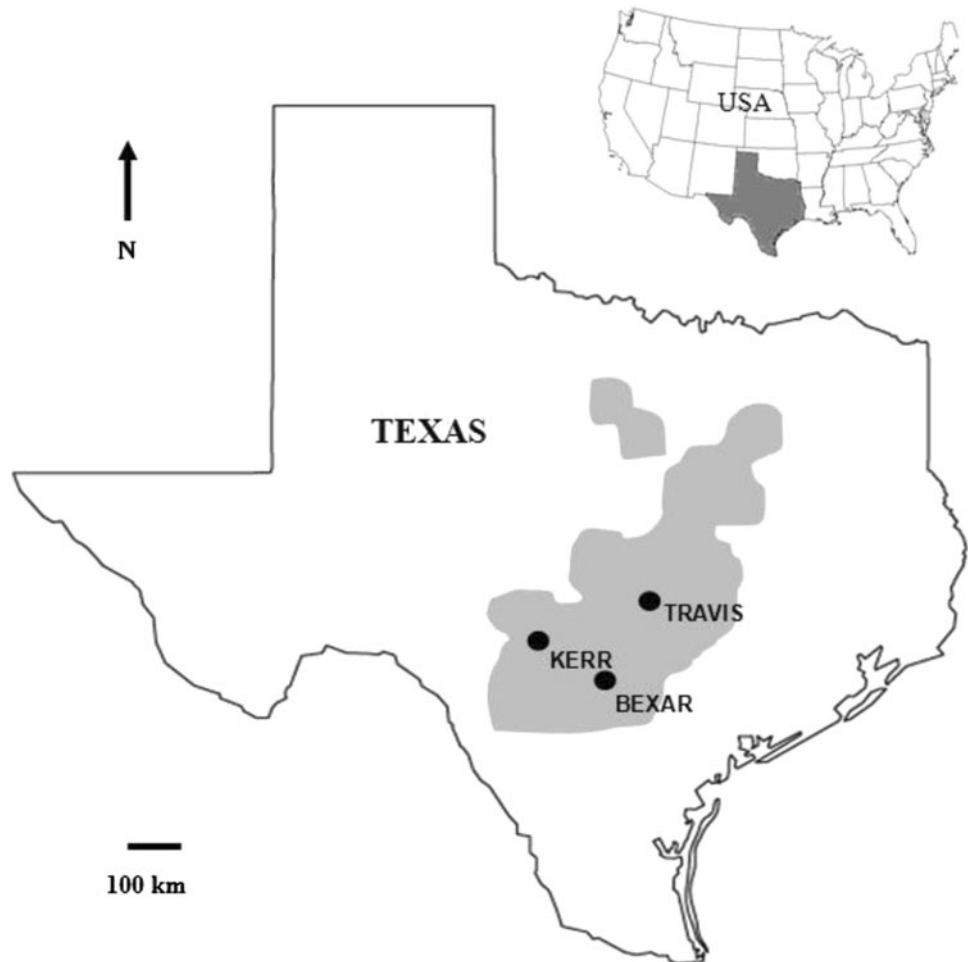
### Locations and times of samples for temporal analyses

Our study focused on temporal samples collected from multiple locations between the years 1890 and 2008. This sampling window provided the greatest extent of temporal separation between samples and also brackets the period during which extensive habitat loss and fragmentation is documented to have taken place. Due to limited archived material available in museum collections, sampled locations correspond to only three (Fig. 1) of seven populations studied in Lindsay et al. (2008). We categorized comparisons of genetic diversity and effective population size within and between time points into two classes—historical sample comparisons, and historical-contemporary comparisons (Table 1). We refer to pre-decline samples from 1890 to 1915 as the historical samples, while samples from 2005 are referred to as the contemporary samples. Historical sample locations correspond to the same county from which contemporary samples were collected except for Bexar, which comprised samples from Bexar–Comal–Kendall (<50 km radius). The contemporary Bexar and Travis samples correspond to the CAB and BAC samples from Lindsay et al. (2008), respectively. Samples labeled 1900 for Kerr consist of samples collected 1895–1902, whereas samples labeled 1890 for Bexar consist of samples collected 1885–1895.

### Archived samples and processing

We followed the guidelines of Bonin et al. (2004) in processing of historical and contemporary samples to minimize genotyping errors. Historical samples were obtained from four national ornithological collections, namely the National Museum of Natural History (NMNH), Washington, DC, USA, the Museum of Comparative Zoology (MCZ), Cambridge, MA, the Field Museum of Natural History (FMNH), Chicago, IL and the American Museum of Natural History (AMNH), New York, NY (sample IDs are provided in Appendix 2). A total of 73 specimens were sampled and from each archived specimen, a small piece (1 mm<sup>3</sup>) of skin was extracted from the toe-pads (Ellegren 1991) with a sterile razor blade and stored in a sterile microcentrifuge tube. DNA extraction was performed using a QiaAmp Microextraction kit (Qiagen, Valencia, CA), with initial overnight digest, followed by spin-column DNA isolation and final elution in 40 µl low-TE buffer. Samples were stored in low-TE buffer at –20°C to prevent degradation of potentially fragile, low-yield DNA extracts until genotyping. All archived sample processing was performed in a separate lab under a sterile hood.

**Fig. 1** Map showing the distribution of breeding habitat for the *D. chrysoparia* in central Texas. Though the shaded region covers the extent of the current breeding range, available breeding habitat is actually relatively rare and is highly fragmented. Filled ovals represent sites where both historical and contemporary samples have been procured



**Table 1** Sample locations, time points and sample sizes used for temporal analysis of changes in genetic diversity, population differentiation and estimation of the effective population size  $N_e$

Sampling site	Sample year	Sample size ( $N$ )
Bexar	1890	11
	2005	17
Kerr	1900	9
	1915	33
	2005	31
Travis	1913	16
	2005	17

#### Establishing reliability and estimating error rates

We first established a framework to quantify the reliability of our genotype dataset using the program RELIOTYPE (Miller et al. 2002). This program uses a maximum-likelihood approach for reducing genotyping errors and provides recommendations for replications at each locus to reach a  $\geq 95\%$  reliability level (the minimum acceptable level). Once individual loci-sample combinations reach or

exceed the reliability threshold, the data can be considered to have a negligible rate of error. We initially determined consistent amplification if at least three out of four attempts resulted in the expected Polymerase Chain Reaction (PCR) product. We used MICROCHECKER (Van Oosterhout et al. 2004) to check our data for genotyping errors, large-allele dropout and null alleles. For loci that passed these initial criteria, we analyzed the data in RELIOTYPE to determine if and how many replicates were necessary to meet a minimum reliability level of 95% (for each locus). Amplification of loci that did not meet the reliability threshold were replicated for the recommended number of times and analyzed again to confirm that they exceeded the 95% threshold. In the final dataset, we retained only loci with average reliability scores equaling or greater than 98%. We also used the program GIMLET (Valière 2002) to estimate the error rate arising due to allelic dropout across all replicates.

Each sample was genotyped following amplification by PCR at each of nine microsatellite loci previously characterized for this species (Lindsay et al. 2008), followed by fragment analysis on an ABI 3130 Genetic Analyzer. PCR



optimization and genotyping analyses were performed as reported in Lindsay et al. (2008). PCR on DNA from archived samples was optimized by changes to MgCl<sub>2</sub> concentrations or with thermoprofile variations.

### Statistical analysis

We collected allelic data following fragment analyses using GENESCAN software (version 3.1, Applied Biosystems, Foster City, CA). We performed exact tests for deviations from Hardy-Weinberg expectations on allele frequencies using the online tool GENEPOP ON THE WEB (Raymond and Rousset 1995). The program GENETIX (Belkhir et al. 2004) was used to estimate measures of unbiased expected heterozygosity ( $H_{EXP}$ ), and the program FSTAT (Goudet 2002) was used to estimate allelic richness ( $A_R$ ).  $A_R$  controls for biases in allelic diversity arising from unequal sample sizes (Leberg 2002). Global diversity indices and other sample-by-locus statistics are provided in Appendix 1.

To quantify the changes in genetic diversity between the historical and contemporary samples, we used a paired  $t$ -test (PROC TTEST, Statistical Analysis Software, SAS Institute, Cary, NC), by locus, to compare  $H_{EXP}$  and  $A_R$  between time points.

Historical population genetic structure was characterized by estimating pairwise  $F_{ST}$  among the historical samples from Kerr, Bexar, and Travis populations in GENETIX (Belkhir et al. 2004). The significance of  $F$ -statistic estimates was assessed using a resampling approach (3,000 permutations) to account for differences in population sample sizes. Identical analyses were performed on the corresponding contemporary populations. To verify that obtained  $F_{ST}$  estimates are not an artifact of high intra-population diversity, we also compared differentiation using two other estimator, namely  $G'_{ST}$  and  $D_{EST}$  (Hedrick 2005; Jost 2008). We used a paired (by locus)  $t$ -test to determine if the degree of pairwise-differentiation was different between the historical and contemporary periods. Additionally, elevated linkage disequilibrium has been associated with genetic fragmentation (Zartman et al. 2006) and we tested whether the differentiation we observed also caused elevated linkage disequilibrium.

### Estimation of $N_e$

We used the pseudo-maximum likelihood estimator (Wang 2001; Wang and Whitlock 2003) as implemented in the program MLNE to estimate  $N_e$  (hereafter referred to as  $MLN_e$ ). MLNE provides estimates of the variance  $N_e$  across time-spans separating two or more sampling intervals (Wang and Whitlock 2003). We estimated  $N_e$  jointly with migration rate ( $m$ ), where it is assumed that migrants

originate from an infinitely large population. Estimation of  $m$  requires that a source population be specified from which gene flow may occur. For each of the three populations for which we estimated  $MLN_e$ , we specified a source population comprised of samples combined from the remaining two populations. We used this estimator as it is the only available temporal estimator that considers populations open to migration, and as we were specifically interested in the temporal estimate of  $N_e$ . For comparison with the  $MLN_e$  estimates, we also estimated the moment  $N_e$  ( $MtN_e$ ) based on equations 15 and 18 from Nei and Tajima (Nei and Tajima 1981), which assumes closed populations.

Estimation of  $N_e$  using either the moment or likelihood approach requires an assumption of discrete generations. As *D. chrysoparia* do not conform to the assumption of discrete generations, we used two different generation lengths ( $T = 1$  and 2 years) to evaluate the effects that  $T$  may have on estimates of  $N_e$ . The majority of adult females in songbirds breed in their first year, whereas reports indicate that age of males at first breeding may be either one or two year (USFWS 1992). Considering this, either generation length may be appropriate, and we applied both in separate analyses in addition to the average of the two (1.5 year/generation).  $MLN_e$  estimates obtained from  $T = 1$  and  $T = 2$  were different, but were qualitatively similar, with results for  $T = 1.5$  being intermediate of the two. Therefore, only results from  $T = 1$  are reported in the main text (Table 3). Estimates for  $T = 2$  are presented in Table S1.

Current census population sizes ( $N_c$ ) for the three sites are approximately 90 (Frels Jr 2006), 485 (Source: Camp Bullis, TX) and 1,000 (USFWS 2004) pairs, for Kerr, Bexar, and Travis, respectively (Lindsay et al. 2008). Census methodologies vary between sites and are subject to minor yearly variations, hence population sizes are not to be considered to be exact and are reported as 3-year averages.

### Results

Approximately 78% of PCR amplifications on archived samples amplified on the first attempt, compared to 98% for the contemporary samples. Consensus genotypes from historical samples were obtained following multiple replications (up to six replicates for different loci) based on the recommendations from the program RELIOTYPE. Only eight of the nine loci analyzed were amplified consistently and met the 98% reliability threshold that we had established. The eight polymorphic microsatellite loci (Lindsay et al. 2008) were scored in both historical and contemporary samples, with no evidence of null alleles. Error rates in historical sample genotypes were estimated to be 1.9%

across loci. A total of 95 different alleles were detected in the historical samples, of which 92 were found in contemporary samples. We did not observe significant deviations from Hardy-Weinberg equilibria across loci.

Based on our analyses of temporal changes in genetic variation over a 100-year interval, we found that populations of the *D. chrysoparia* show reduced genetic diversity, increased genetic fragmentation and small effective population sizes compared to historical estimates. There was no difference between 1900 and 1915 estimates of allelic richness ( $A_R$ ) and heterozygosity ( $H_{EXP}$ ) (Fig. 2).

Both  $A_R$  and  $H_{EXP}$  were significantly lower in contemporary samples compared to historical samples from the same sites, with average declines of 20 and 13%, respectively (Fig. 2). Though the two estimates showed similar declines, there was a greater proportional change in  $A_R$ .

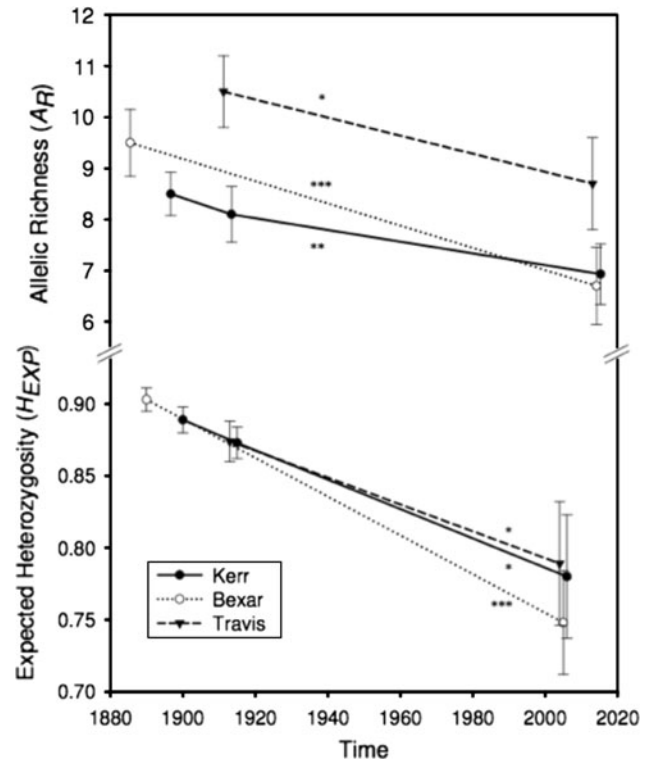
No significant genetic differentiation was detected among the three historical populations in our sample as measured by  $F_{ST}$  (Table 2), and we recovered a similar, albeit slightly different, pattern of differentiation among the three contemporary populations as reported in Lindsay et al. (2008). The most important result, however, was that measures of pairwise differentiation had increased significantly when comparing historic to contemporary levels of differentiation for the same population pairs. This result was retained with other estimators of differentiation ( $G'_{ST}$ ,  $D_{ST}$ ), which control for high genetic diversity within populations (see Appendix 1). We also found that ~56% of pairwise locus tests were significant for linkage disequilibrium.

Across our comparisons,  $MtN_e$  almost always produced estimates larger than  $MLN_e$  (Table 3);  $MtN_e$  estimates of historical  $N_e$  for Kerr (1900–1915) were very large ( $\infty$ ) in all cases (Table 3), whereas the  $MLN_e = 144$  (95% CI 39–6242). The estimated rate of migration was also high in proportion to  $N_e$  (Table 3).

The historical-contemporary estimates of  $N_e$  for Bexar (1890–2005), Travis (1913–2005), and Kerr (1915–2005) were 49 (95% CI 24–212), 273 (95% CI 46–621), and 66 (95% CI 43–419), respectively (Table 3). As our estimates of  $F_{ST}$  indicate that rates of gene flow may not have been constant over the period separating the samples, we are not reporting estimates of  $m$ .

## Discussion

Understanding the genetic impacts of fragmentation is clearly of interest for conservation of species, and the increasing attempts to quantify these impacts in various taxa attest to the exigency of the threats faced by biodiversity (Alo and Turner 2005; Veit et al. 2005; Martinez-Cruz et al. 2007; Born et al. 2008; Jordan and Snell 2008;



**Fig. 2** Estimates of mean genetic diversity (with SEs) for historical and contemporary populations of *D. chrysoparia*. The hypotheses of no difference in estimates of allelic richness ( $A_R$ ) and expected heterozygosity ( $H_{EXP}$ ) were evaluated with a  $T$ -test ( $\alpha = 0.05$ ). Symbols above each bar indicate significant pairwise differences (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ )

Kramer et al. 2008; Angelone and Holderegger 2009). Despite these efforts, the evidence for the genetic impacts of fragmentation is not unequivocal. Our study addresses some of the lingering questions by analyzing genetic changes from samples straddling a period of well documented habitat loss and fragmentation in central Texas. Our results follow similar reports of reduced genetic diversity, increased spatial structuring or low effective population sizes in various taxa, resulting from habitat fragmentation (Ellis et al. 2006; Chevolut et al. 2008; Dixo et al. 2009). However, our study is among the few that were able to report a decline by comparison with pre-fragmentation samples. Our results add to the important body of literature on the effects of fragmentation on conservation genetics and are potentially informative for future management of the golden-cheeked warbler.

### Changes in diversity

Large losses of  $A_R$  are characteristic of bottlenecks (Leberg 1992) and are of concern because random loss of alleles might reduce adaptive potential. Although the decline in



**Table 2** Pairwise differentiation ( $F_{ST}$ ) between pairs of sites that were sampled for both historical and contemporary periods

Sample pair	Historical samples		Contemporary samples		Historical v/s contemporary comparison	
	$F_{ST}$	$P$	$F_{ST}$	$P$	$T$	$P$
Bexar–Kerr	0.00053	0.485	0.031	<0.01	16.46	<0.001
Travis–Bexar	0.00093	0.436	0.051	<0.01	12.85	<0.001
Travis–Kerr	0.000095	0.455	0.009	0.05	19.40	<0.001

Within each time period, we tested the hypothesis that pairwise  $F_{ST}$  estimates would not be significantly different from zero, the expectation for panmictic populations. For temporal comparisons, we used paired  $T$ -tests to test the hypothesis that pairwise  $F_{ST}$  estimates among populations did not differ between historical and the contemporary periods

**Table 3** Estimation of effective population sizes ( $N_e$ ) using temporal sampling approaches and a generation length of 1 year

Likelihood estimator								
Site	Time	No. gen	Mt $N_e$	ML $N_e$	95% CI	$m$	95% CI	$N_c$
Historical sample comparisons								
Kerr	1900–1915	15	$\infty$	144	39–6242	0.362	0.016–0.899	–
Historical—contemporary comparisons								
Bexar	1890–2005	115	98	49	24–212	0.084	0.019–0.187	970
Kerr	1915–2005	90	326	66	35–725	0.053	0.012–0.365	184
Travis	1913–2005	92	378	273	46–621	0.047	0.014–0.323	2000

Values (with 95% confidence intervals) include Nei and Tajima’s moment estimate (Mt $N_e$ ), Wang’s likelihood estimate (ML $N_e$ ), migration ( $m$ ), contemporary census size ( $N_c$ ).  $N_c$  is not available for historical Kerr populations

heterozygosity was not to the same extent as allelic richness, these two indices change at different rates in response to demographic changes, suggesting that population sizes may have been low for a sufficient period of time for inbreeding to accumulate (Nei et al. 1975; Frankham 1995c) in *D. chrysoparia* populations. Losses of  $A_R$  are expected to occur rapidly following bottlenecks compared to changes in  $H_{EXP}$  (Young et al. 1996). Recent population estimates of *D. chrysoparia* populations suggest that declines may have slowed, as a result of which declines in  $A_R$  may have ceased, although it is possible that losses of heterozygosity are ongoing, given the slower rate at which heterozygosity responds to population size changes (Nei et al. 1975). Similar declines in genetic diversity have been documented in other species (Ellis et al. 2006; Lage and Kornfield 2006), but establishing a link with fragmentation has not always been straightforward. Perhaps the most striking finding of our study is its occurrence in combination with increased genetic differentiation.

#### Increased differentiation

We obtained slightly different results compared to Lindsay et al. (2008) partly as a result of increased sample size for the Kerr population (31 instead of 16) and using

one fewer loci than in the previous study. Additionally, all samples were reanalyzed and scored on a different genotyping platform compared to Lindsay et al. (2008). It is also possible that the partitioning of variation would be different when using different numbers of populations in the analysis (a total seven populations were surveyed in that case). Our results suggest reduced gene flow in contemporary populations compared to historical times indicating that barriers to gene flow are recent phenomena. Additionally, our finding of elevated linkage disequilibrium, providing additional evidence for fragmentation. Historical accounts of habitat from central Texas at turn of the 20th century indicate that preferred *D. chrysoparia* habitat may have been more contiguous than today (Schmidly and Sansom 2002), and rapid urbanization of central Texas since the 1940s (including 225,000 km of road construction) is known to have greatly reduced the extent and quality of this songbird’s habitat (USFWS 1992). In a recent review on the genetics impacts of landscape alteration we report that changes in population structure appear to be more common in fragmented populations than losses of genetic diversity (Leberg et al. 2009). The finding of increased differentiation along with losses of diversity is one of the main findings of our study, and was achieved mainly by the availability of temporal samples.

## Effective size estimates

We found relatively low estimates of  $N_e$  based on two different estimators, but they are difficult to interpret without accurate knowledge of census size. The relationship between  $N_e$  and  $N_c$  is much debated in literature (Frankham 1995b; Nunney 1995), but an  $N_e$  that is significantly lower than  $N_c$  indicates that a bottleneck may have occurred and may negatively impact genetic variation over time; thus this ratio is of obvious importance to species conservation (Nunney 2000). We did not estimate  $N_e/N_c$  ratios in *D. chrysoparia* as there is little information about historical census sizes, making such a comparison impossible. The relatively small estimates of  $N_e$  observed in our study may be a consequence of various demographic factors related to habitat loss and fragmentation, and may reflect poor survival in habitat edges (Lindsay et al. 2008), reduced nest success in degraded habitats (Peak 2007; Reidy et al. 2009), high levels of brood parasitism and nest predation (Sperry et al. 2008), or limitation of habitat on wintering grounds (Rappole et al. 2003). A follow-up temporal analysis of the managed populations will be required to assess if and how  $N_e$  is changing in response to management efforts.

Alternatively, *D. chrysoparia* populations may not have been very large historically. Although forested areas are presumed to have been more contiguous in historical times than at present, it is debatable whether suitable habitat was extensive (given the highly specialized habitat requirements). In a scenario where *D. chrysoparia* would have historically existed at relatively small effective sizes, continuing fragmentation might significantly impact genetic diversity. The observed steep increase in genetic differentiation indicates that fragmentation becomes critical for populations that are not large to begin with, and

suffer further declines due to habitat loss. This suggests that gene flow may be much more sensitive to habitat changes than to within population demographic events (Leberg et al. 2009). This apparent loss of connectedness among populations is expected when habitat becomes fragmented and remnant populations become isolated on patches of preserved habitat—the current situation with *D. chrysoparia*.

Our findings of a significant decline in genetic diversity, and increases in genetic differentiation are an important addition to the data on fragmentation effects on vertebrate species. Given the high degree of habitat specialization of *D. chrysoparia* and the slow recovery of habitat to climax oak-juniper communities, our results elicit additional concern for the species' long-term viability. Considering that our earlier study on contemporary populations failed to detect many of the genetic changes that have occurred in the last century, this study emphasizes the importance of temporal sampling for characterizing genetic trends in endangered species.

**Acknowledgments** We thank the AMNH, New York, NY, MCZ Harvard University, Cambridge, MA, FMNH, Chicago, IL, and NMNH, Washington DC for tissue samples. We thank Ft. Hood, Kerr WMA, Balcones Canyonlands NWR, TPWD, K. Barr, C. Goates, J. Hernandez, S. Pathikonda, and L. Butler for access to field sites, and help with field sampling. We thank J. Neigel, S. Mopper and D. Johnson and anonymous reviewers for comments on an earlier version of this manuscript. This study was funded by U.S. Department of Defense under the Section 6.1 Basic Research Program and U.S. Army 6.2 Threatened and Endangered Species Program to RFL and PLL.

## Appendix 1

See Tables 4, 5, 6 and 7

**Table 4** Historical population summary of diversity indices

Gene diversity per locus and population			
Locus1	0.945	0.954	0.941
Locus2	0.898	0.881	0.927
Locus3	0.852	0.854	0.877
Locus4	0.846	0.827	0.886
Locus5	0.873	0.846	0.923
Locus6	0.867	0.919	0.891
Locus7	0.866	0.848	0.877
Locus8	0.865	0.879	0.905
	0.8765	0.876	0.903

**Table 4** continued

Number of alleles sampled				
Locus1	19	18	12	22
Locus2	11	10	12	12
Locus3	9	9	7	9
Locus4	9	9	8	10
Locus5	10	8	10	10
Locus6	11	11	8	11
Locus7	12	10	9	14
Locus8	13	10	10	16
	11.75	10.625	9.5	
Allelic richness per locus and population based on min. sample size of: 11 diploid individuals				
Locus1	13.084	14.283	12	13.063
Locus2	9.185	9.047	12	9.359
Locus3	7.202	7.875	7	7.232
Locus4	7.491	7.851	8	7.573
Locus5	8.282	7.236	10	8.148
Locus6	8.279	9.885	8	8.379
Locus7	8.154	8.123	9	8.111
Locus8	9.003	8.845	10	9.196
	8.835	9.1431	9.5	

**Table 5** Nei's estimation of heterozygosity

LocName	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'	Gis
Locus1	0.888	0.947	0.945	-0.002	-0.003	0.944	-0.002	-0.004	0.062
Locus2	0.855	0.902	0.902	0	0	0.902	0	0	0.052
Locus3	0.831	0.861	0.862	0	0.001	0.862	0	0.001	0.035
Locus4	0.917	0.853	0.853	0.001	0.001	0.853	0.001	0.001	-0.076
Locus5	0.868	0.88	0.874	-0.007	-0.01	0.87	-0.007	-0.011	0.014
Locus6	0.883	0.892	0.883	-0.009	-0.013	0.878	-0.01	-0.015	0.009
Locus7	0.917	0.864	0.854	-0.009	-0.014	0.85	-0.011	-0.017	-0.062
Locus8	0.912	0.882	0.878	-0.005	-0.007	0.875	-0.005	-0.008	-0.034
Overall	0.884	0.885	0.881	-0.004	-0.006	0.879	-0.004	-0.007	0.001

**Table 6** Modern population's summary of diversity

Gene diversity per locus and population			
Loc1		0.93	0.956
Loc2		0.759	0.807
Loc3		0.721	0.704
Loc4		0.532	0.563
Loc5		0.829	0.836

**Table 6** continued

## Gene diversity per locus and population

Loc6	0.841	0.752	0.733
Loc7	0.841	0.868	0.785
Loc8	0.822	0.843	0.893
	0.784	0.7815	0.7846

## Number of alleles sampled

Loc1	16	19	19
Loc2	7	8	6
Loc3	6	6	6
Loc4	5	6	5
Loc5	8	10	8
Loc6	8	9	9
Loc7	7	11	7
Loc8	8	9	11
	8.125	9.75	8.875

## Allelic richness per locus and population based on min. sample size of: 17 diploid individuals

Loc1	15.228	14.511	19
Loc2	6.789	6.902	6
Loc3	5.878	5.461	6
Loc4	4.789	5.059	5
Loc5	7.789	8.319	8
Loc6	7.789	7.72	9
Loc7	6.895	9.315	7
Loc8	7.877	8.458	11
	7.877	8.2181	8.875

**Table 7** Diversity measures

Locus	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'	Gis
Loc1	0.898	0.918	0.938	0.02	0.03	0.948	0.021	0.031	0.022
Loc2	0.79	0.77	0.768	-0.002	-0.003	0.767	-0.002	-0.004	-0.025
Loc3	0.813	0.73	0.767	0.037	0.055	0.785	0.048	0.07	-0.113
Loc4	0.581	0.561	0.566	0.005	0.008	0.569	0.01	0.014	-0.035
Loc5	0.852	0.829	0.827	-0.002	-0.003	0.827	-0.002	-0.003	-0.028
Loc6	0.77	0.775	0.808	0.032	0.048	0.824	0.04	0.059	0.007
Loc7	0.813	0.831	0.872	0.04	0.06	0.892	0.046	0.068	0.022
Loc8	0.87	0.853	0.855	0.003	0.004	0.856	0.003	0.005	-0.02
Overall	0.798	0.783	0.8	0.017	0.025	0.808	0.021	0.031	-0.02

## Appendix 2

Sample ID	Museum
A382291	AMNH
A382292	AMNH
A382293	AMNH
A382294	AMNH
A382295	AMNH
A382296	AMNH
A382297	AMNH
A382360	AMNH
A382361	AMNH
A382362	AMNH
A382363	AMNH
A382364	AMNH
A382365	AMNH
A382305	AMNH
A382310	AMNH
A382313	AMNH
A382318	AMNH
A382323	AMNH
A382327	AMNH
A382329	AMNH
A382335	AMNH
A382340	AMNH
A382341	AMNH
A382346	AMNH
A382348	AMNH
A382351	AMNH
A382352	AMNH
A507004	AMNH
F149870	FMNH
F149871	FMNH
F149872	FMNH
F26172	FMNH
F308240	FMNH
F308244	FMNH
M101045	MCZ
M101254	MCZ
M204935	MCZ
M214364	MCZ
M28413	MCZ
M326084	MCZ
M326086	MCZ
M320914	MCZ
M320923	MCZ
M320940	MCZ
M320941	MCZ
M320943	MCZ
M320946	MCZ

## Appendix continued

Sample ID	Museum
M320956	MCZ
M320957	MCZ
M320964	MCZ
M320965	MCZ
M320967	MCZ
M320968	MCZ
M320971	MCZ
M382341	MCZ
M382364	MCZ
M382380	MCZ
N141771	NMNH
N152408	NMNH
N592677	NMNH
N133176	NMNH
N150528	NMNH
N152520	NMNH
N152527	NMNH
N153056	NMNH
N153057	NMNH
N181808	NMNH
N184442	NMNH
N184833	NMNH

## References

- Alo D, Turner TF (2005) Effects of habitat fragmentation on effective population size in the endangered Rio Grande silvery minnow. *Conserv Biol* 19:1138–1148
- Angelone S, Holderegger R (2009) Population genetics suggests effectiveness of habitat connectivity measures for the European tree frog in Switzerland. *J Appl Ecol* 46:879–887
- Belkhir K, Borsa P, Chiki L, Raufaste N, Bonhomme F (2004) Genetix 4.05. logiciel sous windows TM pour la genetique des populations. Laboratoire genome, populations, interactions, CNRS UMR 5000. Universite de Montpellier II, Montpellier
- Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. *Mol Ecol* 13:3261–3273
- Born C, Hardy OJ, Chevallier MH, Ossari S, Atteke C, Wickings J, Hossaert-Mckey M (2008) Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. *Mol Ecol* 17:2041–2050
- Chevolot M, Ellis JR, Rijnsdorp AD, Stam WT, Olsen JL (2008) Temporal changes in allele frequencies but stable genetic diversity over the past 40 years in the Irish sea population of *Thornback ray*, *raja clavata*. *Heredity* 101:120–126
- Crow JF, Denniston C (1988) Inbreeding and variance effective population numbers. *Evolution* 42:482–495
- Dixo M, Metzger JP, Morgante JS, Zamudio KR (2009) Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal forest. *Biol Conserv* 142:1560–1569



- Ellegren H (1991) DNA typing of museum birds. *Nature* 354:113
- Ellis JS, Knight ME, Darvill B, Goulson D (2006) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae). *Mol Ecol* 15:4375–4386
- Frankham R (1995a) Conservation genetics. *Annu Rev Genet* 29:305–327
- Frankham R (1995b) Effective population-size adult-population size ratios in wildlife—a review. *Genet Res* 66:95–107
- Frankham R (1995c) Inbreeding and extinction: a threshold effect. *Conserv Biol* 9:792–800
- Frels D Jr (2006) Performance report. Kerr Wildlife Management Area, Hunt, p 8
- Goudet J (2002) FSTAT 2.9.3.2, a program to estimate and test gene diversities and fixation indices. <http://www.unil.ch/izea/software/fstat.html>. Accessed 25 April 2010
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59:1633–1638
- Howeth JG, McGaugh SE, Hendrickson DA (2008) Contrasting demographic and genetic estimates of dispersal in the endangered coahuilan box turtle: a contemporary approach to conservation. *Mol Ecol* 17:4209–4221
- Jordan MA, Snell HL (2008) Historical fragmentation of islands and genetic drift in populations of galapagos lava lizards (*Microlophus albemarlensis* complex). *Mol Ecol* 17:1224–1237
- Jost L (2008)  $G_{ST}$  and its relatives do not measure differentiation. *Mol Ecol* 17:4015–4026
- Kramer AT, Ison JL, Ashley MV, Howe HF (2008) The paradox of forest fragmentation genetics. *Conserv Biol* 22:878–885
- Kroll JC (1980) Habitat requirements of the golden-cheeked warbler: management implications. *J Range Manag* 33:60–66
- Lage C, Kornfield I (2006) Reduced genetic diversity and effective population size in an endangered Atlantic salmon (*Salmo salar*) population from Maine, USA. *Conserv Genet* 7:91–104
- Leberg PL (1992) Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* 46:477–494
- Leberg PL (2002) Estimating allelic richness: Effects of sample size and bottlenecks. *Mol Ecol* 11:2445–2451
- Leberg PL, Athrey GNR, Barr KR, Lindsay D, Lance R (2009) Implications of landscape alteration for the conservation of genetic diversity of endangered species. In: DeWoody JA, Bickham JW, Michler C, Nichols K, Rhodes OE, Woeste K (eds) *Molecular insights into natural resource conservation and management*. Cambridge University Press, Cambridge, p 392
- Lindsay DL, Barr KR, Lance R, Tweddle SA, Hayden TJ, Leberg PL (2008) Habitat fragmentation and genetic diversity of an endangered, migratory songbird, the golden-cheeked warbler (*Dendroica chrysoparia*). *Mol Ecol* 17:2122–2133
- Martinez-Cruz B, Godoy JA, Negro JJ (2007) Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. *Mol Ecol* 16:477–486
- Miller CR, Joyce P, Waits LP (2002) Assessing allelic dropout and genotype reliability using maximum likelihood. *Genetics* 160:357–366
- Nei M, Tajima F (1981) Genetic drift and estimation of effective population-size. *Genetics* 98:625–640
- Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* 29:1–10
- Nichols RA, Bruford MW, Groombridge JJ (2001) Sustaining genetic variation in a small population: evidence from the mauritius kestrel. *Mol Ecol* 10:593–602
- Nunney L (1995) Measuring the ratio of effective population-size to adult numbers using genetic and ecological data. *Evolution* 49:389–392
- Nunney L (2000) The limits to knowledge in conservation genetics—the value of effective population size. *J Evol Biol* 32:179–194
- Peak RG (2007) Forest edges negatively affect golden-cheeked warbler nest survival. *Condor* 109:628–637
- Rappole JH, King DI, Diez J (2003) Winter- vs. breeding-habitat limitation for an endangered avian migrant. *Ecol Appl* 13:735–742
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Reidy JL, Thompson FR, Peak RG (2009) Factors affecting golden-cheeked warbler nest survival in urban and rural landscapes. *J Wildl Manag* 73:407–413
- Rodriguez-Munoz R, Mirol PM, Segelbacher G, Fernandez A, Tregenza T (2007) Genetic differentiation of an endangered capercaillie (*Tetrao urogallus*) population at the southern edge of the species range. *Conserv Genet* 8:659–670
- Sato T, Harada Y (2008) Loss of genetic variation and effective population size of Kirikuchi charr: implications for the management of small, isolated salmonid populations. *Anim Conserv* 11:153–159
- Schmidly DJ, Sansom A (2002) Texas natural history: a century of change, 1st edn. Texas Tech University, Lubbock
- Segelbacher G, Manel S, Tomiuk J (2008) Temporal and spatial analyses disclose consequences of habitat fragmentation on the genetic diversity in capercaillie (*Tetrao urogallus*). *Mol Ecol* 17:2356–2367
- Sperry JH, Peak RG, Cimprich DA, Weatherhead PJ (2008) Snake activity affects seasonal variation in nest predation risk for birds. *J Avian Biol* 39:379–383
- USFWS (1992) Golden-cheeked warbler (*Dendroica chrysoparia*) recovery plan. U.S. Fish and Wildlife, Albuquerque, p 97
- USFWS (2004) Biological opinion, consultation number: 2-12-05-F-021. U.S. Fish and Wildlife Service, Pennsylvania
- Valiére N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Mol Ecol Notes* 2:377–379
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Veit ML, Robertson RJ, Hamel PB, Friesen VL (2005) Population genetic structure and dispersal across a fragmented landscape in cerulean warblers (*Dendroica cerulea*). *Conserv Genet* 6:159–174
- Wang J (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genet Res* 78:243–257
- Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 163:429–446
- Yamamoto S, Morita K, Koizumi I, Maekawa K (2004) Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. *Conserv Genet* 5:529–538
- Young A, Clarke GM (2000) Conclusions and future directions: what do we know about the genetic and demographic effects of habitat fragmentation and where do we go from here? In: Young A, Clarke GM (eds) *Genetics, demography and viability of fragmented populations*, 1st edn. Cambridge University Press, Cambridge, pp 361–366
- Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol Evol* 11:413–418
- Zartman CE, McDaniel SF, Shaw AJ (2006) Experimental habitat fragmentation increases linkage disequilibrium but does not affect genetic diversity or population structure in the Amazonian liverwort *Radula flaccida*. *Mol Ecol* 15:2305–2315