Conserv Genet (2008) 9:1633–1638
DOI 10.1007/s10592-008-9518-0

SHORT COMMUNICATION

Limited genetic structure and evidence for dispersal among populations of the endangered Florida grasshopper sparrow, *Ammodramus savannarum floridanus*

Kristin A. Mylecraine · Natalie L. Bulgin · H. Lisle Gibbs · Peter D. Vickery · Dustin W. Perkins

Received: 12 April 2007 / Accepted: 9 January 2008 / Published online: 29 January 2008

Abstract The Florida grasshopper sparrow, *Ammodramus savannarum floridanus*, is a non-migratory, endangered subspecies endemic to the prairie region of south-central Florida. It has experienced significant population declines and is currently restricted to five locations. We found substantial levels of variation in microsatellites and mtDNA control region sequences, estimates of inbreeding genetic effective population sizes that were much larger than the estimated census size, and no evidence of inbreeding within five sampled populations ($n = 105$). We also found a lack of genetic structure among populations ($F_{ST} = 0.0123$ for microsatellites and $\theta = 0.008$ for mtDNA), and evidence for dispersal between populations, with 7.6% of all individuals identified as immigrants to their population of capture. We suggest that the subspecies be managed as a single management unit on a regional scale rather than as multiple management units on a local subpopulation scale. There is still a limited opportunity to preserve much of the present genetic variation in this subspecies, if immediate measures are taken to reverse the current population decline before this variation is reduced by genetic drift.

Keywords Dispersal · Endangered species · Assignment test · Conservation genetics · Effective population size · Immigration

K. A. Mylecraine (✉) · H. L. Gibbs
Department of Evolution, Ecology and Organismal Biology, The Ohio State University, 300 Aronoff Laboratory, 318 West 12th Ave., Columbus, OH 43210, USA
e-mail: mylecraine.1@osu.edu

N. L. Bulgin
Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON, Canada L8S 4K1

P. D. Vickery
Center for Ecological Research, Richmond, ME 04357, USA

D. W. Perkins
Department of Biology, Mesa State College, Northern Colorado Plateau Network, National Park Service, 1100 North Ave., Grand Junction, CO 81501, USA

Introduction

The endangered Florida grasshopper sparrow, *Ammodramus savannarum floridanus*, is endemic to the prairie region of south-central Florida. It is geographically isolated from the eastern subspecies, *A. s. pratensis* by at least 500 km (AOU 1983) and differs by its non-migratory habit and morphology. Historic records suggest that this subspecies was locally abundant in south-central Florida (Delany et al. 1985); however, habitat destruction and fragmentation during the last century have led to severe population declines and range contractions (Delany et al. 1985; Delany and Cox 1986). Six known Florida grasshopper sparrow populations occurred in south-central Florida at the time of this study (Vickery and Perkins 2003), including three populations on Avon Park Air Force Range, and single populations at Three Lakes Wildlife Management Area, Kissimmee Prairie State Preserve, and the Ordway-Whittell Kissimmee Prairie Sanctuary. Subsequent to this study, one of the Avon Park populations (Bravo Range) steadily declined and no birds were detected in 2003 (Vickery and Perkins 2003). The Ordway-Whittell site was acquired as an addition to Kissimmee Preserve, a prairie corridor was restored to connect the sites, and they are now considered a single population. One additional population was located on private land in Okeechobee County, for a total of five current populations (Vickery

and Perkins 2003; Perkins and Vickery 2005). In 2003, surveys indicated a population size of 379 adult males, and a total population of 992 birds, including juveniles (Vickery and Perkins 2003).

Previous genetic work by our group (Bulgin et al. 2003) compared the genetic characteristics of *A. s. floridanus* as a whole with non-endangered subspecies of grasshopper sparrows. Here, we expand on these findings by quantifying inter-population variation and dispersal between individual Florida grasshopper sparrow populations to provide guidance for management and conservation efforts. Our specific objectives are to (1) examine levels of genetic diversity within populations, to determine whether population decline has resulted in loss of genetic diversity, (2) examine the genetic structure of existing populations to identify any genetically distinct management units among groups of populations, and (3) to use individual assignment tests to identify inter-population dispersers and obtain a quantitative measure of connectivity between populations.

Methods

Sample collection and genetic methods

Samples were collected and genotyped for six microsatellite loci and 879 bp of the mtDNA control region as described previously (Bulgin et al. 2003). Briefly, we sampled 105 Florida grasshopper sparrows from five of the six known locations between 1995 and 1998 (Fig. 1). Sample sizes ranged from 11 to 34 per population (Table 1). We were unable to obtain sufficient sample sizes from the sixth population, Bravo Range; however, this population is now thought to be extinct (Vickery and

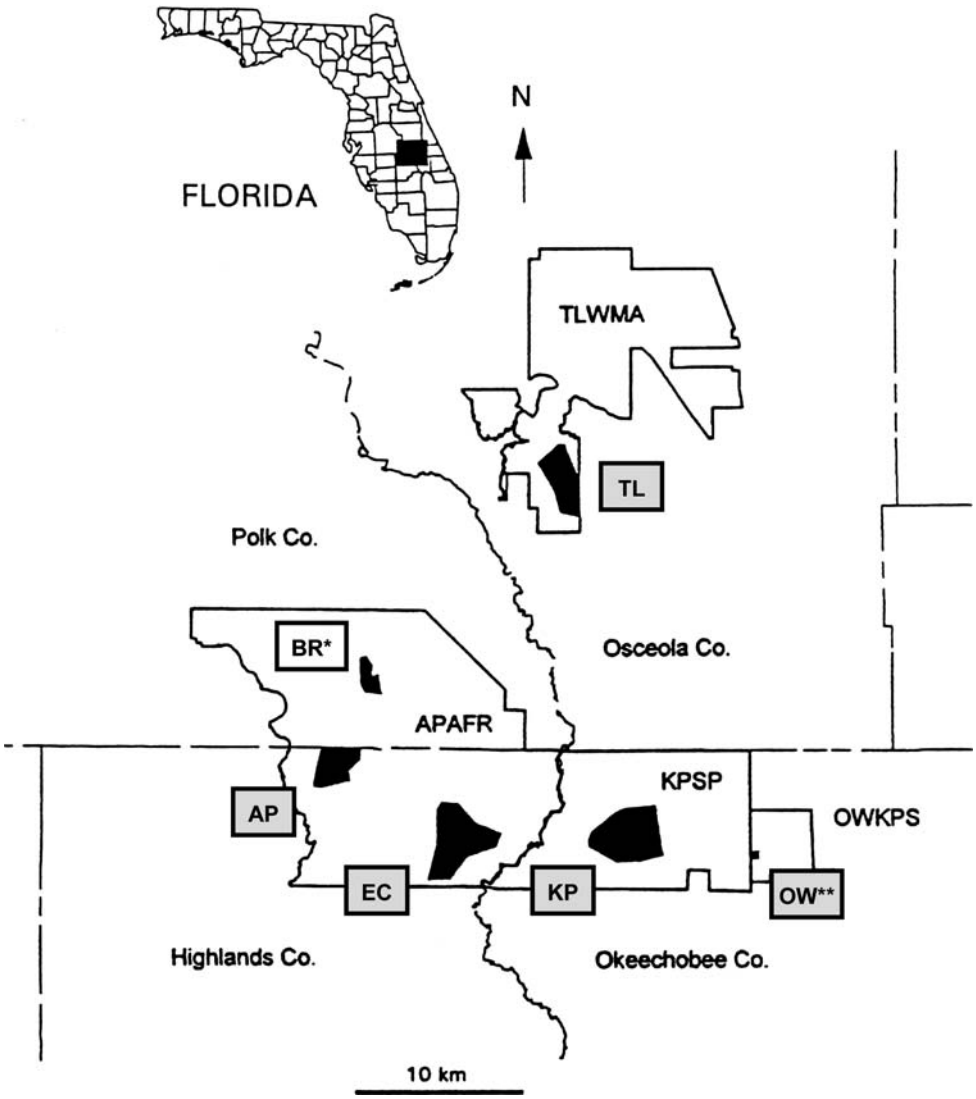

Fig. 1 Locations of known Florida grasshopper sparrow populations during study period (1995–1998). Three populations are located on the Avon Park Air Force Range: Delta/OQ Range (AP), Echo Range (EC), and Bravo Range* (BR—not sampled). Additional populations are located at Three Lakes Wildlife Management Area (TL), Kissimmee Prairie State Preserve (KP), and Ordway-Whittell Kissimmee Prairie Sanctuary** (OW). The blackened area in the state map represents the area encompassed by the more detailed map showing the individual populations. *This site was occupied during the study period, but the population here has subsequently declined and no birds were detected in 2003 (Perkins and Vickery 2005). **This site was subsequently acquired as an addition to Kissimmee Prairie Preserve and a prairie corridor connecting the two populations has been restored

Table 1 Genetic diversity statistics for five Florida grasshopper sparrow populations based on (a) six microsatellite loci and (b) mtDNA control region sequences

Pop.	N	N_e^a	N_{census}^b	Mean # alleles	H_o	H_e	F_{IS}
(a) Microsatellites							
AP	34	2,839 (832–12,985)	4 (11)	10.3 ± 4.4	0.73 ± 0.17	0.73 ± 0.13	0.01 ± 0.09
OW	11	2,624 (397–27,334)	n/a[c]	6.8 ± 3.7	0.67 ± 0.27	0.72 ± 0.21	0.11 ± 0.22
KP	19	3,652 (339–34,654)	206 (575)	10.0 ± 4.2	0.77 ± 0.18	0.76 ± 0.21	−0.04 ± 0.10
EC	16	3,348 (787–27,334)	8 (20)	8.8 ± 4.4	0.69 ± 0.14	0.75 ± 0.18	0.07 ± 0.07
TL	25	2,431 (571–15,278)	138 (328)	8.8 ± 3.2	0.67 ± 0.17	0.71 ± 0.16	0.07 ± 0.09

(b) *mtDNA*

Pop.	n	Haplotypes		Pairwise differences	k	π
		Total	Private			
AP	33	15	6	4.477	0.922	0.005
OW	9	9	3	6.000	1.000	0.007
KP	19	13	6	5.554	0.959	0.006
EC	13	10	4	4.795	0.962	0.005
TL	24	13	7	3.804	0.931	0.004

For microsatellites, values are given for the number of birds genotyped (n), evolutionary effective population size (N_e), and census population size (N_{census}), and mean values (±SD) are given for mean number of alleles per locus, observed (H_o) and expected (H_e) heterozygosities and inbreeding coefficient (F_{IS}). For mtDNA, values are given for the number of birds genotyped (n), number of haplotypes observed, type and number of polymorphic sites, haplotypic diversity (k) and nucleotide diversity per site (π)

[a] Effective population size (Lehmann et al. 1998), calculated based on average H_e (range for individual loci)

[b] Number of adult males (total population size) estimated from point count surveys. Total population size estimated based on a stable age distribution (Vickery and Perkins 2003)

[c] This population historically supported 10–15 breeding pairs, but was extirpated due to high water tables. Three singing males were again detected in 2002 after a corridor was established connecting OW and KP (Perkins and Vickery 2005)

Perkins 2003). Four individuals were removed from the mtDNA analysis because of poor PCR amplification.

Data analysis

Levels of variability and effective population size

We calculated the number of mtDNA haplotypes, haplotypic diversity (k), and nucleotide diversity per site (π) for each sample using ARLEQUIN 2.000 (Schneider et al. 2000). For the microsatellite data, we calculated the mean number of alleles per locus, observed (H_o) and expected (H_e) heterozygosity, and inbreeding coefficient (F_{IS}, Weir and Cockerham 1984) using GENEPOP (Raymond and Rousset 1995). We estimated long-term inbreeding effective population size from expected heterozygosity, assuming a step-wise mutation model (SMM), based on the formula $N_e = ([1/1-H_e]^2-1)/8\mu$ (Lehmann et al. 1998) and a microsatellite mutation rate, $\mu = 5.6 \times 10^{-4}$ (Goldstein et al. 1995). We tested for deviation from Hardy–Weinberg expectations using the HW exact test and the Markov chain method for P-value estimation (Guo and Thompson 1992), and tested for linkage disequilibrium between pairs of loci in GENEPOP (Raymond and Rousset 1995). All α-values were adjusted for multiple comparisons using a sequential Bonferroni correction (Rice 1989).

Population structure

For mtDNA, we calculated both frequency and distance-based F_{ST} values overall and for each population pair using ARLEQUIN 2.000 (Schneider et al. 2000). For microsatellites, we calculated (i) Weir and Cockerham's (1984) theta (θ) using FSTAT 2.9.3.2 (Goudet 2001) and assuming IAM, and (ii) Slatkin's (1995) rho (ρ) using RST CALC 2.2 (Goodman 1997) and assuming SMM.

To further assess population structure with microsatellites, we used two Bayesian methods to identify the optimal number of clusters of individuals (STRUCTURE 2, Pritchard et al. 2000) and populations (BAPS 3.1, Corander et al. 2004). We estimated K using the admixture model and the infer lambda option in STRUCTURE, using a burn-in period of 10^5 iterations, followed by 10^6 iterations. We used ln-likelihood scores to calculate the posterior probability (Pritchard and Wen 2004) for each value of K and chose the value with the highest posterior

probability. Three separate runs were performed for each K to ensure consistency. We also partitioned pre-defined populations into the most probable number of clusters and then assigned populations to each cluster using a Bayesian approach and stochastic optimization in BAPS 3 (Corander et al. 2003). Both methods assume Hardy–Weinberg expectations and linkage equilibrium between loci within populations.

Inter-population dispersal

We used the combined microsatellite and mtDNA dataset and the IMMANC assignment test (Rannala and Mountain 1997) to identify immigrants, defined as individuals captured in one population, but having genotypes that were significantly more likely to originate from another population. We chose IMMANC because it provides a rarely available estimate of the power of each test to detect an immigrant if one is present.

Results and discussion

Levels of variability and effective population size

Florida grasshopper sparrow populations have retained substantial variability in both mtDNA control region sequences and microsatellites and have not experienced significant inbreeding, despite historical population decline and fragmentation. As described previously (Bulgin et al. 2003), 37 unique mtDNA haplotypes were identified. Two haplotypes (GRSP7 and GRSP22) were found in all five samples, and 26 (70%) haplotypes were found in only one sample (Table 1). For microsatellites, the mean number of alleles per locus ranged from 6.8 ± 3.7 for OW to 10.3 ± 4.4 for AP. Mean observed heterozygosity ranged from 0.67 ± 0.27 for OW and 0.67 ± 0.17 for TL to 0.77 ± 0.18 for KP, and expected heterozygosity ranged from 0.71 ± 0.16 for TL to 0.76 ± 0.21 for KP. There was no evidence of significant inbreeding in any sample (Table 1). We found no evidence of linkage disequilibrium for any pairs of loci in any population, and no departures from Hardy–Weinberg expectations for any population or locus, except for locus Asμ15 in the TL population. Estimates of long-term inbreeding effective population sizes are much larger than the census population size. Estimates of N_e ranged from 2,431 for TL to 3,652 for KP. Summing across samples, we estimate total $N_e = 14{,}894$. The census population size is estimated to be 992 birds (Vickery and Perkins 2003), yielding $N_e/N_{census} = 15.0$. We suggest that populations have maintained substantial amounts of variability and large effective population sizes in the face of population declines, because population declines have occurred recently and insufficient time has elapsed for genetic drift to erode genetic variation.

Population structure

We found little population genetic structure among populations using either microsatellites ($\theta = 0.008$; 95% CI: 0.002–0.015) or mtDNA ($F_{ST} = 0.0123$; $P = 0.0811$). Two population pairs showed significant differentiation in microsatellites ($P < 0.05$) assuming an IAM model, with $\theta = 0.0397$ for the comparison of KP and AP, and $\theta = 0.0279$ for EC and TL. Assuming a SMM model, only one estimate was significant ($\rho = 0.0121$ between AP and TL). No population pairs showed significant differentiation in mtDNA, using either frequency or distance-based approaches. Neither of the Bayesian clustering methods detected hidden population structure. BAPS grouped all five populations into a single cluster (log ml $= -2521.6$), and STRUCTURE grouped all individuals together ($K = 1$), with a posterior probability of 1.0 in three separate runs.

Genetic measures of dispersal

The lack of genetic structure suggests ongoing gene flow or recent separation of populations (c.f. Bulgin et al. 2003). Assignment tests suggest ongoing gene flow between these populations. Despite a lack of significant structure, the power to detect immigrants using IMMANC approached 1.00 for individual analyses (range 0.953–0.995, mean $=$ 0.976, SE $= 0.001$) likely due to small but significant differences in allele frequencies between populations not reflected in population-wide measures of differentiation. Eight immigrants (7.6%) were identified (Table 2). All 11 birds from OW originated in that population, and no birds from OW were identified in any other population. The remaining four populations received and donated migrants. Four sparrows captured at AP (11.8% of all birds captured) were immigrants, two originated from EC, one from KP, and one from TL. One bird from EC originated from AP, two birds captured at KP originated from AP, and one moved from KP to TL. We recognize the possibility that individuals might be erroneously identified as dispersers, given similar allele frequencies among populations and the lack of genetic structure; however, we feel that this is unlikely, because of the high power estimates for all analyses. Power generally increases with increasing population genetic structure (Rannala and Mountain 1997; Cornuet et al. 1999); however, a high degree of power can be obtained with low differentiation (Rannala and Mountain 1997).

Table 2 Results of the IMMANC individual assignment tests for five Florida grasshopper sparrow populations, including the number (above) and percentage (below) of birds assigned to each population, and the number of immigrants to and emigrants from each population

		Assigned to (emigrated from)					#
		AP	EC	OW	KP	TL	Immigrants
Population of capture (immigrated to)	AP	*30*	2	0	1	1	4
		0.882	0.059	0.000	0.029	0.029	(11.8%)
	EC	1	*15*	0	0	0	1
		0.063	*0.938*	0.000	0.000	0.000	(6.3%)
	OW	0	0	*11*	0	0	0
		0.000	0.000	*1.000*	0.000	0.000	(0.0%)
	KP	2	0	0	*17*	0	2
		0.105	0.000	0.000	*0.895*	0.000	(10.5%)
	TL	0	0	0	1	*24*	1
		0.000	0.000	0.000	0.040	*0.960*	(4.0%)
#		3	2	0	2	1	
Emigrants		(8.8%)	(12.5%)	(0.0%)	(10.5%)	(4.0%)	

Banding studies provide limited support for dispersal between populations (Delany et al. 1995; Dean et al. 1998; Vickery and Perkins 2003). Only one of 50 nestlings banded in one study was resighted in subsequent years (Perkins and Vickery 2001). Delany et al. (1995) found one male that moved 2 km from his natal site to a breeding territory the following year, and Miller (2006) captured an adult bird at KP in 2003 that was banded as a hatching year juvenile at AP in 1996. These banding records, combined with our genetic results, suggest some degree of connectivity among populations; however, it is not clear whether this level of dispersal is adequate to maintain genetic variation and prevent inbreeding. As a general rule, 1–10 migrants per generation may be sufficient (Mills and Allendorf 1996; Wang 2004). Dispersal among current populations appears to be above this one migrant threshold; however, our analyses do not address actual gene movement between populations, because the fate and reproductive success of these dispersers in unknown.

Conservation implications

The lack of genetic structure and evidence of dispersal between Florida grasshopper sparrow populations indicate that the subspecies should be managed regionally as a single management unit rather than locally as multiple independent subpopulations. Despite severe population declines and habitat fragmentation, populations have not yet experienced significant losses of genetic diversity. There is still an opportunity to preserve much of the genetic variation present in these populations, but this will require immediate measures to stop the current population decline and increase population numbers, before this variation is reduced by genetic drift.

Acknowledgements We thank T. Dean for help in obtaining samples, L. DeSousa, O. Haddrath and M. Vallianatos for assistance in the lab and B. N. White for comments. This research was funded by a contract from the U.S. Fish and Wildlife Service, Vero Beach, Florida.

References

American Ornithological Union [AOU] (1983) Checklist of North American birds, 6th edition. American Ornithologists Union, Washington, DC

Bulgin NL, Gibbs HL, Vickery P et al (2003) Ancestral polymorphisms in genetic markers obscure detection of evolutionarily distinct populations in the endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*). Mol Ecol 12:831–844

Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. Genetica 163: 367–374

Corander J, Waldmann P, Marttinen P et al (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics 20:2363–2369

Cornuet J-M, Piry S, Luikart G et al (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000

Dean TF, Delany MF, Chapman EW et al (1998) Longevity and site fidelity of Florida grasshopper sparrows. J Field Ornithol 69: 51–54

Delany MF, Cox JA (1986) Florida grasshopper sparrow breeding distribution and abundance in 1984. Fla Field Nat 14:100–104

Delany MF, Moore CT, Progulske DR Jr (1995) Territory size and movements of Florida grasshopper sparrows. J Field Ornithol 66:305–309

Delany MF, Stevenson HM, McCracken R (1985) Distribution, abundance, and habitat of the Florida grasshopper sparrow. J Wildlife Manage 49:626–631

Goldstein DB, Ruiz-Linares A, Cavalli-Sforza LL et al (1995) Genetic absolute dating based on microsatellites and the origin of modern human. Proc Natl Acad Sci USA 92:6723–6727

Goodman S (1997) RST CALC: a collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. Mol Ecol 6:881–885

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportions for multiple alleles. Biometrics 48:361–372

Lehmann T, Hawley WA, Grebert H et al (1998) The effective population size of *Anopheles gambiae* in Kenya: implications for population structure. Mol Biol Evol 15:264–276

Miller P (2006) Long distance dispersal of a Florida Grasshopper Sparrow. Fla Field Nat 33:123–124

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. Conserv Biol 10:1509–1518

Perkins DW, Vickery PD (2001) Annual survival of an endangered passerine, the Florida grasshopper sparrow. Wilson Bull 113: 211–216

Perkins DW, Vickery PD (2005) Effects of altered hydrology on the breeding ecology of the Florida grasshopper sparrow and Bachman's sparrow. Fla Field Nat 33:29–40

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard JK, Wen W (2004) Documentation for *structure* software: Version 2. Available online: http://pritch.bsd.uchicago.edu/structure.html Cited 18 Oct 2006

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94:9197–9201

Raymond M, Rousset F (1995) GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Schneider S, Roessli D, Excoffier L (2000) Arlequin ver. 2.000

Vickery PD, Perkins DW (2003) Florida grasshopper sparrow population viability analysis and reserve design. Final Report to the U.S. Fish and Wildlife Service, Vero Beach, FL

Wang J (2004) Application of the one-migrant-per-generation rule to conservation and management. Conserv Biol 18:332–343

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370