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Tools and Technology Article

Using 2 Genetic Markers to Discriminate Among Canada Goose Populations in Ohio

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ABSTRACT Canada goose (*Branta canadensis*) harvest management depends on reliable estimates of harvest composition, and established genetic methods provide an alternative to traditional methods. We expanded upon previous genetic studies by comparing the utility of 6 nuclear microsatellite loci and mitochondrial (mtDNA) control region sequences for discriminating among giant (*B. c. maxima*) and interior (*B. c. interior*) populations in Ohio (USA) Canada goose harvests at both individual and population levels. Subspecies and populations exhibited greater differentiation in mtDNA ($F_{ST} = 0.202$) than microsatellites ($F_{ST} = 0.021$), as would be expected based on differences in effective population size. Neither microsatellites nor mtDNA alone were sufficient for estimating harvest composition at the subspecies or population level in simulations and empirical blind tests using individuals of known origin; however, a combined microsatellite + mtDNA dataset yielded accurate and precise harvest derivations at the subspecies level. Both population-level mixed stock analysis and individual-level assignment tests provided accurate results, but a large proportion of birds could not be assigned with confidence at the individual level. We applied mixed stock analysis and the combined microsatellite + mtDNA dataset to Ohio's 2003–2004 harvest and found that interior populations accounted for 4.9% (95% CI = 1.7–8.0%) of the statewide early season and 9.3% (95% CI = 6.9–11.6%) of the regular and late-season harvested sample. These results suggest that maximum likelihood harvest derivations are highly dependent on the choice of genetic markers. Studies should only employ markers that exhibit sufficient variation and have been shown through simulations and empirical testing to accurately discriminate among the subspecies or management populations of interest. (JOURNAL OF WILDLIFE MANAGEMENT 72(5):1220–1230; 2008)

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KEY WORDS *Branta canadensis*, genetic stock identification, giant Canada goose, harvest derivation, harvest management, interior Canada goose, microsatellite DNA, mixed stock analysis, mtDNA.

Canada geese (*Branta canadensis*) have a complicated taxonomy, with 6 subspecies currently recognized (Delacour 1954, Banks et al. 2004). Subspecies can be grouped into those exhibiting long migrations to breed in Arctic and subarctic regions of North America (subarctic-nesting) or those breeding in southern Canada and the United States (temperate-nesting). For harvest management purposes, Canada geese are further divided into a number of management populations; in many cases, one subspecies contains multiple management populations, each representing a discrete breeding population. Harvest management attempts to maintain a desirable and sustainable level of harvest, while ensuring viability of all management populations and maintaining these populations at or near their objective population sizes (North American Waterfowl Management Plan Committee 2004), but this is complicated by the co-occurrence of birds from multiple breeding populations during autumn and winter hunting seasons.

Of the 4 management populations of Canada geese affiliated with the Mississippi Flyway, 3 occur regularly in Ohio harvests (T. Moser, United States Fish and Wildlife Service [USFWS], unpublished data). These include two subarctic-nesting populations of the interior subspecies (*B. c. interior*) and one temperate-nesting population of the giant subspecies (*B. c. maxima*). The Southern James Bay Population (SJBP; *B. c. interior*) breeds on Akimiski Island and in the Hudson Bay Lowlands to the west and south of James Bay, and winters from southern Ontario (Canada) and Michigan to Mississippi, Alabama, and South Carolina (USA). The Mississippi Valley Population (MVP; *B. c. interior*) breeds in northern Ontario in the Hudson Bay Lowlands, west of James Bay and south of Hudson Bay, and winters primarily in Wisconsin, Illinois, and Michigan (USA). Both interior populations have fluctuated, but remained fairly stable, and are currently at or above their population objectives of 100,000 SJBP and 375,000 MVP geese (USFWS 2007). The Mississippi Flyway Giant Population (MFGP; *B. c. maxima*) has been reestablished in all Mississippi Flyway states, and continues to increase, with a current population of 1.6 million (USFWS 2007). Due to discrete interior and giant breeding ranges and high levels of individual philopatry, potential interbreeding between subspecies is unlikely; however, movement between SJBP and MVP management populations has been observed (Leafloor 1998).

Harvest management in Ohio aims to maximize harvest of MFGP geese to control this population and provide recreational opportunities, while limiting the harvest of SJBP and MVP geese. An understanding of the spatial and temporal variation in harvest composition is necessary to identify the appropriate management options to accomplish these goals. Genetic techniques provide a viable alternative to traditional methods of estimating harvest derivations, such as band recovery analyses and morphometric discrimination (Pearce et al. 2000, Inman et al. 2003, Scribner et al.

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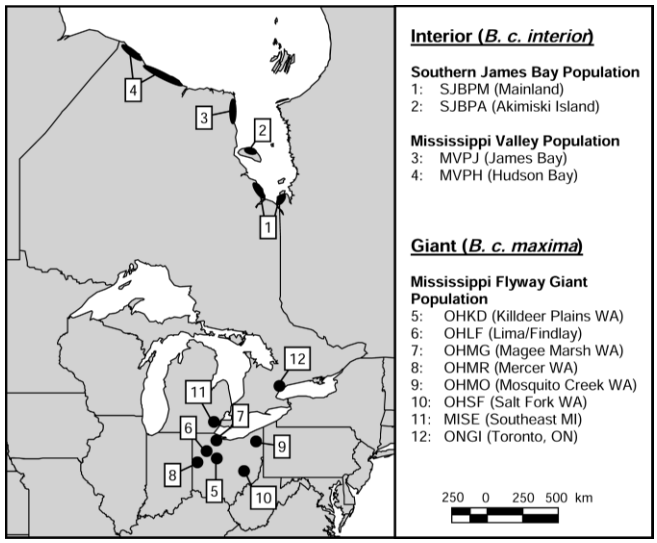

Figure 1. Collection locations of Canada goose baseline samples for genetic analysis. We sampled 8 subpopulations of the giant subspecies (Mississippi Flyway Giant Population), from Ohio and Michigan, USA, and Ontario, Canada, and 4 subpopulations of the interior subspecies (Southern James Bay Population and Mississippi Valley Population), from Ontario and Nunavit, Canada. Ohio collections were made during 2003 and 2004; we obtained all remaining populations from Scribner et al. (2003).

2003, Shorey et al. 2007). Both individual-level and population-level genetic methods have potential for estimating harvest composition. Individual assignment tests use allele frequencies to assign individuals of unknown origin to their most likely source population and provide an estimate of statistical probability for each assignment (Davies et al. 1999). These techniques have not been fully explored for Canada goose populations. At the population level, mixed stock analysis (MSA; Pella and Milner 1987) uses allele frequencies in all potential contributing (baseline) populations and maximum likelihood methods to estimate proportional contributions of each population or subspecies to admixed harvest samples. In Michigan and the Pacific Northwest, MSA has been successful in estimating proportional contributions of Canada goose and cackling goose (*Branta hutchinsii*) subspecies and management populations (Pearce et al. 2000; Inman et al. 2003; Scribner et al. 2003, 2005; Shorey et al. 2007), but these methods have not been rigorously tested in widespread geographic areas, despite potential applicability throughout North America and across all 4 flyways. Most previous studies employing MSA have employed biparentally inherited, nuclear microsatellite loci (Inman et al. 2003, Scribner et al. 2003, Shorey et al. 2007), but maternally inherited mitochondrial DNA (mtDNA) variation has also shown sufficient discriminatory power in some applications (Pearce et al. 2000).

Here we expand on previous genetic studies by comparing the utility of nuclear microsatellites and mtDNA control-region sequences for accurately estimating the composition of Ohio Canada goose harvests, using both population-level mixed stock analysis and individual assignment tests. Our specific objectives were to 1) quantify the extent of genetic structure among temperate-nesting giant populations (MFGP) and subarctic-nesting interior populations (MVP and SJBP) using microsatellites and mtDNA control region sequences, 2) compare the ability of microsatellites and mtDNA to accurately estimate harvest composition at subspecies and management population levels, using both MSA and individual assignment tests, and 3) apply these techniques to determine the composition of Ohio's 2003–2004 harvest.

STUDY AREA

Mixed stock analysis and assignment methods assume that all potential populations contributing to the unknown harvest mixture have been sampled. We included in our baseline the 3 management populations likely to occur in Ohio harvests (T. Moser, unpublished data). We sampled geese from 4 subpopulations, representing 2 management populations of the interior subspecies: MVP along the southern coast of Hudson Bay (MVPH), MVP along the western coast of James Bay (MVPJ), SJBP on Akimiski Island (SJBPA), and SJBP on the mainland coast of southern James Bay (SJBPM). We also sampled 8 subpopulations of the Mississippi Flyway Giant Population of the giant subspecies: Killdeer Plains Wildlife Area (OHKD), Magee Marsh Wildlife Area (OHMG), Mercer Wildlife Area (OHMR), Mosquito Creek Wildlife Area (OHMO), Lima and Findlay area (OHLF), and Salt Fork Wildlife Area (OHSF), Ohio; southeastern Michigan (MISE); and Toronto, Ontario (ONGI; Fig. 1).

METHODS

Sample Collection

We collected primary and secondary blood-feather quills from flightless interior and giant Canada geese during banding operations. We sampled only prefledgling goslings at all sites to avoid sampling molt migrants, nonbreeding individuals from temperate-nesting populations that migrate northward and often co-occur with subarctic-nesting populations during summer molt (Abraham et al. 1999), and we sampled individuals from multiple drives to maximize variation and avoid sampling relatives. We collected Ohio samples in June 2003 and June 2004. We obtained from a previous study Michigan, Ontario, and interior samples, which were collected prior to 2003 using the same precautions to avoid sampling molt migrants (Inman et al. 2003, Scribner et al. 2003). We suspended blood quills in lysis buffer and stored them at −20° C. Sample sizes for individual populations ranged from 44 for ONGI to 100 for MVPH.

We collected a second blind sample from known interior and giant subpopulations in 2005, including individuals from OHKD, OHMG, OHMO, OHMR, OHSF, SJBPA, SJBPM, and MVPH subpopulations. We collected samples following the same protocol stated above for baseline samples, and we used them to create mixtures of known interior:giant proportions to evaluate the accuracy of mixed stock analysis. Subspecies and population information was

unknown to lab personnel. Sample sizes ranged from 29 for SJBPM to 73 for SJBPA.

We implemented a statewide collection system, where ≥5 selected hunters from each county provided primary feathers from geese harvested during 2003–2004. Our total statewide sample included 1,526 geese, about 2% of the total harvest (87,946; USFWS 2005). Using these samples, we compared likelihood estimates of harvest composition using the 3 genetic datasets, microsatellites, mtDNA, and the combined microsatellite + mtDNA database.

Genetic Methods

We extracted DNA from blood quill and feather samples using the DNEasy Tissue Kit (Qiagen, Valencia, CA). We selected 6 microsatellite loci, including 5 loci used in previous studies of Canada goose harvest composition (Scribner et al. 2003): Bcaμ7, Bcaμ9, Bcaμ11, Hhiμ1 (Buchholz et al. 1998), and TTUCG-1 (Cathey et al. 1998), and one additional locus, TTUCG-5 (Cathey et al. 1998). We performed polymerase chain reaction (PCR) in 10-μl reactions containing 6.5 μl ddH_2O, 1 μl ancient buffer (20 μl 25 mM $MgCl_2$, 50 μl 1M Tris pH 8.0, 500 μl KCl, 217.5 μl ddH_2O, 50 μl Tris pH 8.8, 100 μl gelatin, and 62.5 μl BSA), 0.2 μl 10 μM dNTPs, 0.7 μl 50 mM $MgCl_2$, 0.1 μM Taq (5 U/μl), and 0.25 μl of forward and reverse primer (10 μM). We end-labeled forward primers with fluorescent dyes HEX (Bcaμ7 and Hhiμ1), FAM (Bcaμ9 and Bcaμ11), and NED (TTUCG-1 and TTUCG-5). We performed amplifications using an initial incubation at 94° C for 2 minutes, followed by 35 cycles of 94° C for 1 minute, annealing temperature for 30 seconds, and 72° C for 45 seconds, and a final extension at 72° C for 5 minutes. Annealing temperatures were 63° C for Bcaμ7, 64° C for Bcaμ9, 60° C for Bcaμ11, 61° C for Hhiμ1, 46° C for TTUCG-1, and 56° C for TTUCG-5. We multiloaded diluted PCR products, combined them with 9.7 μl formamide and 0.3 μl ROX 350 size standard (Applied Biosystems, Foster City, CA, USA), and ran them on a 3100 Genetic Analyzer (Applied Biosystems).

We used the C1 and C1R primers of Sorenson and Fleischer (1996) to sequence a 387-bp fragment of the 5′ end of the mitochondrial DNA control region (3′ end of domain I; Baker and Marshall 1997). These primers have previously been tested to confirm that they do not amplify nuclear sequences of mitochondrial origin (Pierson et al. 2000). We PCR-amplified products in 15-μl reaction volumes, consisting of 7.02 μl ddH_2O, 1.5 μl 10× Buffer or ancient buffer, 1.5 μl BSA, 0.8 μl 10 μM dNTPs, 1.5 μl $MgCl_2$, 0.08 μl Taq (5 U/μl), and 0.8 μl of each primer. Amplifications included an initial incubation at 80° C for 5 minutes, followed by 40 cycles of 94° C for 1 minute, 60° C for 1 minute, 72° C for 1 minute, and a final extension at 72° C for 5 minutes. We precipitated PCR-products using a Polyethylene Glycol/EtOH procedure, dried them in a vacuum centrifuge, and then resuspended them in 20 μl ddH_2O. We carried out cycle sequencing using the C1R primer and the Big Dye version 3.1 Cycle Sequencing Kit (Applied Biosystems). We cleaned cycle-sequenced products and sequenced them on a 3700 Genetic Analyzer (Applied Biosystems) at the Plant-Microbe Genomics Facility at The Ohio State University. We edited sequences using ALIGNER (CodonCode, Dedham, MA, USA) then manually aligned sequences and designated haplotypes using BIOEDIT (Hall 2005).

Data Analysis

We selected 32 samples from the 2003–2004 harvest to calculate our error rate for microsatellite genotyping and mtDNA sequencing. We reextracted these samples independently, from a second feather from the same bird, genotyped and sequenced them blindly, and compared results with the original genotypes. We defined microsatellite error rate as the percentage of allelic differences relative to the total number of allelic comparisons (Bonin et al. 2004), and we defined mtDNA error rate as the percentage of individuals that had different haplotypes in 2 runs.

We identified and binned microsatellite alleles using GENOTYPER and GENEMAPPER software (Applied Biosystems). Mixed stock analysis and the analysis of genetic differentiation require assumptions of Hardy–Weinberg equilibrium and independence among all microsatellite loci. To test for deviations from Hardy–Weinberg equilibrium for each locus–population combination, we used the exact test and the Markov chain method to estimate without bias the exact *P*-value, implemented in GENEPOP (Guo and Thompson 1992, Raymond and Rousset 1995). We tested for linkage disequilibrium among loci within each population using GENEPOP (Raymond and Rousset 1995).

We used ARLEQUIN (Schneider et al. 2000) to calculate F_{ST} values for each subpopulation pair, for microsatellite loci and for mtDNA control region sequences, based on haplotype frequencies. We used a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) to partition the total genetic variation in both sets of genetic markers into 3 components: between subspecies (interior vs. giant), among subpopulations within subspecies, and within subpopulations. We adjusted all *P*-values for multiple comparisons using a sequential Bonferroni correction (Rice 1989).

We used 2 methods to test our ability to accurately estimate proportional contributions of interior and giant Canada geese to Ohio harvests: 1) baseline simulations and 2) blind tests using individuals of known origin. We performed simulations using the simulation mode in Program SPAM, v. 3.7 (Debevec et al. 2000, Alaska Department of Fish and Game 2003). We used SPAM to create simulated populations containing user-specified mixtures of baseline populations, and then we performed MSA analysis on the simulated populations to calculate the maximum likelihood proportional estimates of each management population (MFGP, MVP, and SJBP) and subspecies (interior and giant) and compared this to the known value to assess the ability of MSA to accurately estimate proportional contributions to unknown harvest mixtures. We performed a series of 100% simulations,

defined as simulated populations ($n = 200$) composed of 100% of each the 12 baseline subpopulations. We also performed a number of simulated interior:giant mixtures with known interior proportions ranging from 0% to 100%. To assess the effect of mixture sample size on the accuracy of our estimates, we used 3 sample sizes ($n = 200$, $n = 50$, and $n = 20$). To compare the 2 sets of genetic markers, we performed simulations on 3 separate datasets: microsatellites, mtDNA, and a combined microsatellites + mtDNA dataset. We modeled baseline allele frequency distributions using the Rannala–Mountain baseline posterior (Rannala and Mountain 1997), which allows for estimation of baseline allele frequencies for loci with many low-frequency alleles, and calculated maximum likelihood estimates of stock composition in Program SPAM. We used 10,000 bootstrap resamplings to calculate mean proportional contribution estimates for each management population or subspecies and 95% symmetric bootstrap confidence intervals.

We used the 2005 blind sample from known interior and giant subpopulations as a second method to test the accuracy of MSA estimates. First, we performed 100% blind tests, a series of analyses conducted on subsets containing 100% composition of each of the 8 known subpopulations, and we used SPAM to estimate proportional contributions of each subspecies and management population. The true composition was 100% giant for all 5 giant subpopulations and 100% interior for all 3 interior subpopulations, and we expected the maximum likelihood estimates to be equivalent. Second, we used these samples to create interior:giant mixtures ($n = 200$) with interior proportions varying from 0% to 100% and compared the estimated values to the true mixture proportions. We compared blind test results using microsatellites alone, using mtDNA alone, and using the combined microsatellite + mtDNA dataset.

We performed individual assignments using the maximum likelihood classification in the Program MLE (Topchy et al. 2004), which can use both Mendelian (i.e., microsatellite) and nonMendelian (i.e., mtDNA) loci simultaneously to assign individuals to the most likely source population and calculate the posterior probability for each classification. We used an assignment threshold of 0.95; we considered unassigned all individuals with a posterior probability below this threshold. We tested the accuracy of this method for 2 genetic datasets, microsatellites alone and the combined microsatellite + mtDNA dataset, assigning baseline samples of known origin to both subspecies (giant or interior) and management population (MFGP, MVP, or SJBP). We used the leave-one-out strategy, in which we excluded the individual of interest from baseline allele frequency calculations, and determined the number of individuals that were assigned to the correct subspecies or management population and the number that were incorrectly assigned or unassigned. We also assigned the 2005 blind samples, from known interior and giant subpopulations, to subspecies and management population, using both genetic datasets, and determined the proportion of individuals correctly assigned.

We used the 2003–2004 harvest samples to estimate contributions of interior and giant subspecies to Ohio's early, regular, and late seasons, statewide and in each of the state's 3 Canada goose zones (Ohio Department of Natural Resources Division of Wildlife 2006). The 2003–2004 early season was held statewide 1–15 September. The regular season extended from 18 to 26 October and 7 December to 1 January in the Lake Erie Zone; from 18 October to 29 November and 7 December to 2 January in the North Zone; and from 25 October to 16 November and 13 December to 28 January in the South Zone. For this analysis, we combined the 2005 blind test samples with our original baseline to increase baseline sample sizes, and we chose mixed stock analysis because a large proportion of geese could not be assigned to a source population at the individual level. We used Program SPAM to calculate maximum likelihood estimates of harvest composition and compared results using 3 different genetic datasets, microsatellites, mtDNA, and the combined microsatellite + mtDNA dataset.

RESULTS

Genotyping Error Rate

The mtDNA haplotype designations were identical for all individuals sequenced in 2 independent runs, resulting in an mtDNA error rate of 0%. We found 11 microsatellite allelic differences out of 384 comparisons, resulting in an error rate of 2.9%. Ten of the erroneous alleles belonged to one individual, suggesting that this individual was either switched during the genotyping process or the 2 feathers supplied by the hunter were not from the same individual. When we removed this individual from the analysis, the error rate was reduced to 0.3%, roughly equivalent to microsatellite error rates from other studies (Bonin et al. 2004).

Population Differentiation

We found no evidence of deviations from Hardy–Weinberg equilibrium for any locus in any of the 12 baseline subpopulations. Tests for linkage disequilibrium revealed that loci were independent in 171 out of 180 tests (95%). Significant linkage occurred in $\geq$1 locus combination in 5 of the 12 baseline subpopulations, but no loci were consistently linked in multiple subpopulations, suggesting an overall lack of disequilibrium.

We found population differentiation using both microsatellites and mtDNA; however, degree of differentiation was much greater in mtDNA than in microsatellites. Using 6 microsatellite loci, overall $F_{ST} = 0.021$ ($P < 0.001$). Pairwise F_{ST} ranged from 0.002 ($P = 0.194$) for the MVPH–MVPJ comparison to 0.049 ($P < 0.001$) for the SJBPA–OHLF and SJBPA–OHMR comparisons (Table 1). Seven comparisons were not significant ($\alpha = 0.05$), including one comparison between 2 giant subpopulations (OHMG–OHMO) and 2 comparisons between 2 interior subpopulations (MVPH–MVPJ and MVPJ–SJBPM). The remaining 4 nonsignificant values were between one giant and one interior subpopulation (OHMG–MVPH, OHMO–

Table 1. Pair-wise F_{ST} values for 12 baseline subpopulations of Canada geese. We sampled 8 subpopulations of the giant subspecies (Mississippi Flyway Giant Population [MFGP]), from Ohio and Michigan (USA), and Ontario, Canada, and 4 subpopulations of the interior subspecies (Southern James Bay Population [SJBP] and Mississippi Valley Population [MVP]), from Ontario and Nunavut (Canada). Ohio collections were made during 2003 and 2004; we obtained all remaining populations from Scribner et al. (2003). F_{ST} values based on mitochondrial DNA (mtDNA) control region sequences are given above the diagonal, and values based on 6 microsatellite loci are given below the diagonal. Bolded entries represent inter-subspecies comparisons.

	N		Giant subspecies (F_{ST})								Interior subspecies (F_{ST})			
Population[a]	**Microsats**	**mtDNA**	**OHKD**	**OHLF**	**OHMG**	**OHMR**	**OHMO**	**OHSF**	**MISE**	**ONGI**	**MVPH**	**MVPJ**	**SJBPA**	**SJBPM**
OHKD	92	91		0.111	0.057	0.125	0.078	0.186	0.092	0.175	**0.133**	**0.161**	**0.314**	**0.126**
OHLF	84	84	0.009		0.143	0.124	0.132	0.067	0.047	0.253	**0.210**	**0.244**	**0.396**	**0.202**
OHMG	99	99	0.008	0.014		0.201	0.038	0.164	0.073	0.123	**0.100**	**0.119**	**0.280**	**0.094**
OHMR	88	88	0.017	0.006	0.015		0.189	0.256	0.194	0.313	**0.253**	**0.299**	**0.449**	**0.256**
OHMO	86	85	0.011	0.014	0.003[b]	0.015		0.136	0.052	0.079	**0.036**	**0.044**	**0.224**	**0.045**
OHSF	93	90	0.035	0.035	0.020	0.030	0.019		0.018[b]	0.197	**0.194**	**0.217**	**0.366**	**0.185**
MISE	54	55	0.019	0.025	0.009	0.014	0.013	0.014		0.114	**0.105**	**0.122**	**0.267**	**0.097**
ONGI	44	41	0.034	0.027	0.018	0.022	0.013	0.009	0.016		**0.072**	**0.083**	**0.249**	**0.066**
MVPH	100	83	**0.016**	**0.023**	**0.003**[b]	**0.025**	**0.003**[b]	**0.018**	**0.013**	**0.012**		0.005[b]	0.193	0.036
MVPJ	65	63	**0.017**	**0.022**	**0.007**	**0.021**	**0.004**[b]	**0.008**	**0.011**	**0.003**[b]	0.002[b]		0.146	0.036
SJBPA	85	75	**0.038**	**0.049**	**0.030**	**0.049**	**0.027**	**0.024**	**0.036**	**0.027**	0.026	0.011		0.216
SJBPM	72	60	**0.027**	**0.032**	**0.012**	**0.031**	**0.015**	**0.014**	**0.015**	**0.008**	0.005	0.003[b]	0.018	

[a] OHKD = Killdeer Plains Wildlife Area, Ohio (MFGP), OHLF = Lima and Findlay, Ohio (MFGP), OHMG = Magee Marsh Wildlife Area, Ohio (MFGP), OHMR = Mercer Wildlife Area, Ohio (MFGP), OHMO = Mosquito Creek Wildlife Area, Ohio (MFGP), OHSF = Salt Fork Wildlife Area, Ohio (MFGP), MISE = Southeastern Michigan (MFGP), ONGI = Toronto, Ontario (MFGP), MVPH = MVP on Hudson Bay, MVPJ = MVP on James Bay, SJBPA = SJBP on Akimiski Island, SJBPM = SJBP on the mainland along southern James Bay.

[b] Nonsignificant values after sequential Bonferroni correction ($\alpha = 0.05$).

MVPH, OHMO–MVPJ, and ONGI–MVPJ). Using AMOVA, we were able to attribute only 0.61% ($P = 0.023$) of total variation to differences between subspecies and 1.53% ($P < 0.001$) to differences among subpopulations within subspecies. The remaining 97.86% ($P < 0.001$) of variation was attributed to individual variation within subpopulations.

We found greater population differentiation in mtDNA control region sequences, with an overall $F_{ST} = 0.202$ ($P < 0.001$). Pair-wise F_{ST} values ranged from 0.005 ($P = 0.212$) for the MVPH–MVPJ comparison to 0.449 ($P < 0.001$) for the SJBPA–OHMR comparison (Table 1). Only 2 pair-wise comparisons were not statistically significant ($\alpha = 0.05$), including one comparison between 2 giant subpopulations (OHSF–MISE) and one comparison between 2 interior subpopulations (MVPH–MVPJ); all comparisons between subspecies were significant. The AMOVA ascribed 8.66% ($P = 0.006$) of total variation to subspecies differences, 11.51% ($P < 0.001$) to differences among subpopulations within subspecies, and 79.83% ($P < 0.001$) to individuals within subpopulations. We identified 43 haplotypes among the 12 baseline subpopulations (GenBank accession no. EU247035-EU247077).

Mixed Stock Analysis

Both the mtDNA and the combined microsatellite + mtDNA datasets estimated the composition of simulated populations with greater accuracy than did microsatellites alone. Maximum likelihood estimates for the 100% simulations using microsatellites alone ranged from 83.2% to 99.0% of the correct subspecies, when the expected value was 100% for that subspecies. At the management population level, estimates ranged from 73.3% to 99.0% of the correct population. Subspecies estimates ranged from 94.4% to 99.7% for mtDNA and 95.7% to 99.7% for the combined dataset, and management population estimates ranged from 93.3% to 99.7% and 94.8% to 99.7% (Fig. 2).

For simulated mixtures, the true composition was within the 95% confidence intervals for all simulations; however, estimates were more accurate and had smaller confidence intervals using the mtDNA and combined microsatellite + mtDNA datasets than using microsatellites alone (Fig. 3). With a simulated mixture population size of 200, estimates for the combined dataset fell within 0.1–1.4% of the true values (Fig. 3e) and estimates for the mtDNA dataset fell within 0.0–1.6% (Fig. 3c), whereas estimates for microsatellites alone differed 0.1–9.1% from the true values (Fig. 3a). Reducing the mixture sample size to 50 and 20 inflated the confidence intervals and reduced the accuracy of our estimates, particularly when we did not include mtDNA. At a mixture size of 20 individuals, the mtDNA and combined datasets yielded estimates within 0.2–3.1% and 0.2–2.5%, respectively, but the estimates based on microsatellites alone provided estimates that fell only within 0–17.9% of the true value.

Using 100% blind tests and microsatellites alone, large proportions of known subpopulations were incorrectly assigned to subspecies. For 3 giant subpopulations (true composition = 100% giant), estimates of giant proportions were >94%, and the true value fell within the 95% confidence intervals (Fig. 4a). However, much of the remaining 2 giant subpopulations were incorrectly assigned; 44.9% (95% CI = 13.4–76.4%) of OHMO and 13.2% (95% CI = 0.0–34.5%) of OHSF were assigned to the interior subspecies, primarily to the MVP management population (Fig. 4a). The MSA estimates for both of the SJBP interior subpopulations (true composition = 100% interior) were accurate at the subspecies level (>99% interior), but 42.8% (95% CI = 19.9–65.7%) of the MVP

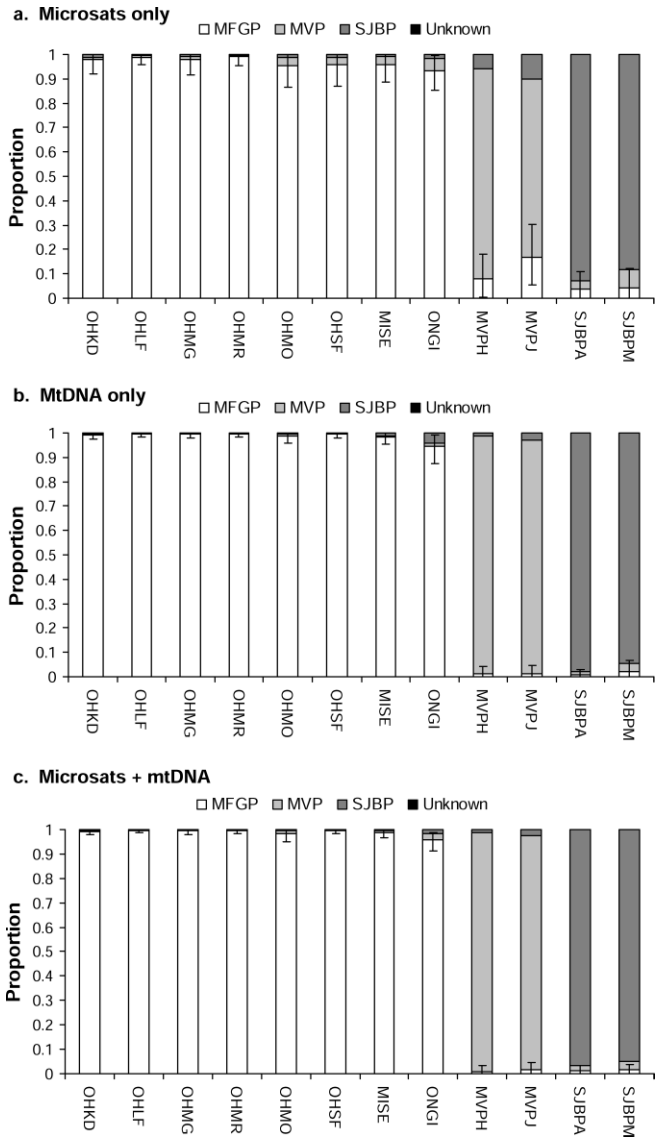

Figure 2. Results of 100% baseline simulations for 12 Canada goose baseline populations. We used Program SPAM to simulate populations (n = 200), each consisting of 100% of 1 of 12 baseline populations, and then we assessed the ability of mixed stock analysis to correctly estimate proportional contributions of each of 3 management populations: Mississippi Flyway Giant Population (MFGP, USA), Mississippi Valley Population (MVP, USA) and Southern James Bay Population (SJBP, Canada), using a) 6 microsatellite loci, b) mitochondrial DNA (mtDNA) control region sequences, and c) microsatellites + mtDNA. We expect to see 100% of the correct management population. Refer to Table 1 and Figure 1 for subpopulation names and locations.

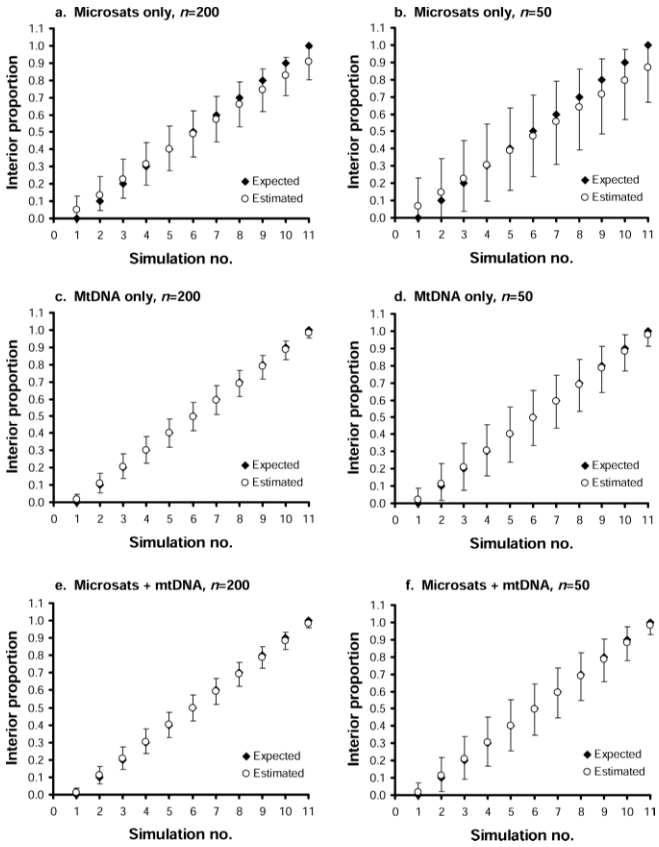

Figure 3. Results of simulated mixture analysis. We used Program SPAM to simulate mixtures of interior and giant Canada geese (n = 200 and 50, respectively), with true interior proportions varying from 0.0 to 1.0. We then performed mixed stock analysis, calculated maximum likelihood estimates of the proportion of interiors in each simulated mixture, and compared estimated values to the true (exp) proportions, using 6 microsatellite loci (a, n = 200; and b, n = 50), mitochondrial DNA (mtDNA) control region sequences (c, n = 200; and d, n = 50), and the combined microsatellite + mtDNA control region dataset (e, n = 200; and f, n = 50).

sample was incorrectly assigned to the giant subspecies. At the management population level, 23.1% (95% CI = 4.9–41.2%) of SJBPA and 26.2% (95% CI = 0.0–60.1%) of SJBPM were assigned to MVP, and 10.6% (95% CI = 0.0–29.7%) of MVPH were assigned to SJBP. Using mtDNA alone, subspecies estimates for 7 populations were within 6.4% of the correct subspecies; however, only 59.3% (95% CI = 16.9–100.0%) of the SJBPM population was assigned correctly to subspecies and 50.8% (95% CI = 18.9–82.7%) to management population (Fig. 4b). Using the combined microsatellite + mtDNA dataset significantly improved the accuracy of MSA estimates (Fig. 4c) at the subspecies level and increased confidence in those estimates. For all 5 giant subpopulations (true composition = 100% giant), maximum likelihood estimates ranged from 95.7% (95% CI = 82.2–100.0%) to 100% giant. For the 3 interior subpopulations (true composition = 100% interior), estimates ranged from 90.6% (95% CI = 79.9–100.0%) interior for MVPH to 100% interior for SJBPA. MSA was not very reliable for distinguishing between the SJBP and MVP management populations, with 9.1% (95% CI = 0.6–17.5%) of SJBPA and 44.1% (95% CI = 16.9–71.2%) of SJBPM assigned to MVP and 6.6% (95% CI = 0.0–22.1%) of MVPH assigned to SJBP.

For a series of known mixtures (interior proportions ranging from 0–100%), the maximum likelihood estimates exhibited greater accuracy and precision using the combined microsatellite + mtDNA dataset than using microsatellites or mtDNA alone. Using microsatellites alone (Fig. 5a; n = 200), MSA overestimated interior proportions by 8–11% for all mixtures with true interior proportions of $\leq$30%. For mixtures with true interior proportions of $\geq$70%, mixed stock analysis underestimated interior proportions by 3.2–

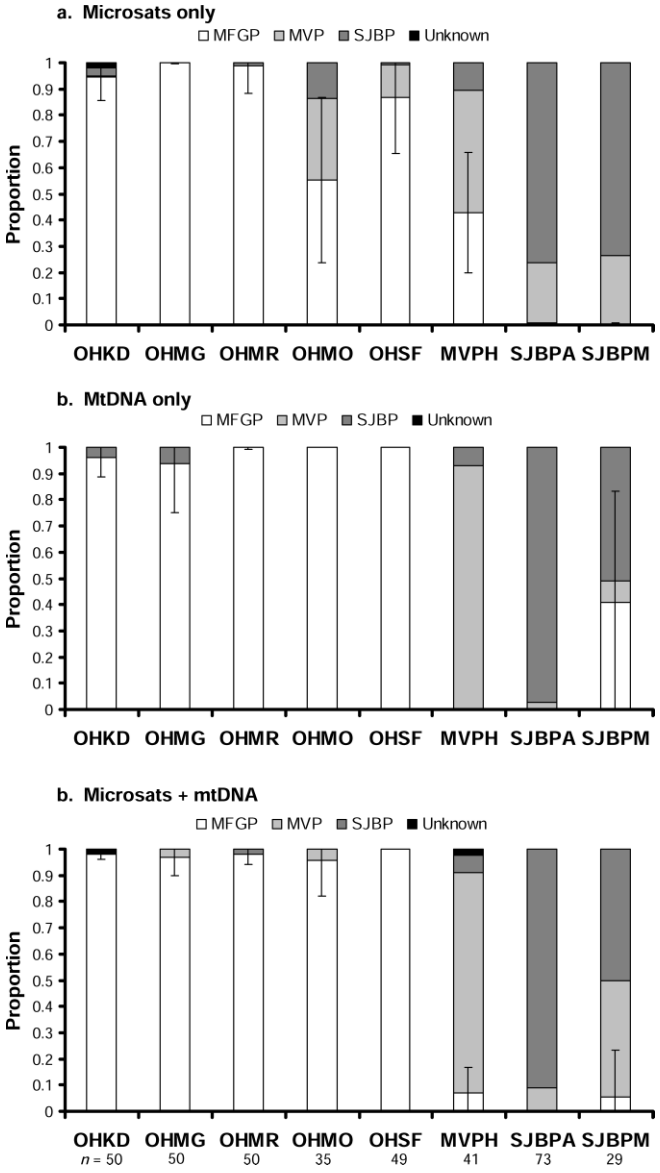

Figure 4. Results of 100% blind tests. We collected Canada goose samples of known origin (in the United States and Canada) during 2005, including 5 Ohio giant subpopulations (OHKD, OHMG, OHMR, OHMO, and OHSF) and 3 interior subpopulations (MVPH, SJBPA, and SJBPM), and used these to assess the ability of mixed stock analysis to accurately assign each subpopulation to the correct management population. We used Program SPAM to calculate the estimated proportion of each management population (Mississippi Flyway Giant Population [MFGP], Southern James Bay Population [SJBP], or Mississippi Valley Population [MVP]) using a) 6 microsatellite loci, b) mitochondrial DNA (mtDNA) control region sequences, and c) the combined microsatellite + mtDNA control region dataset. Refer to Table 1 and Figure 1 for subpopulation names and locations.

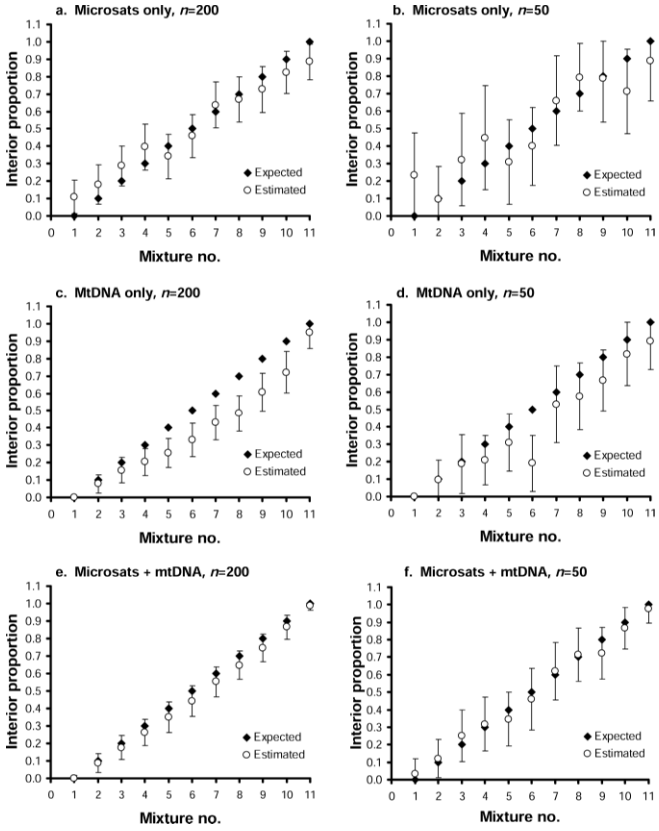

Figure 5. Results of blind test mixture analysis. We collected Canada goose samples of known origin (in the United States and Canada) during 2005, including 5 Ohio giant subpopulations (OHKD, OHMG, OHMR, OHMO, and OHSF) and 3 interior subpopulations (MVPH, SJBPA, and SJBPM). We then created known mixtures with interior proportions ranging from 0.0 to 1.0, performed mixed stock analysis, and compared maximum likelihood estimates with the true (exp) interior proportions, using 6 microsatellite loci (a, $n = 200$; and b, $n = 50$), mitochondrial DNA (mtDNA) control region sequences (c, $n = 200$; and d, $n = 50$) and the combined microsatellite + mtDNA dataset (e, $n = 200$; and f, $n = 50$).

11.4%. The mtDNA alone (Fig. 5c; $n = 200$) underestimated interior proportions by 0.1–21.6%. Using the combined dataset (Fig. 5e), MSA estimates were within 0.2–5.7% of true values. Both microsatellites and mtDNA provided unreliable estimates with smaller mixture sizes, with estimates deviating as much as 23.5% for microsatellites (Fig. 5b) and 31.0% for mtDNA (Fig. 5d) at $n = 50$, and 41.9% and 26.1%, respectively, at $n = 20$. For the combined dataset, estimates remained within 7.8% of the true value at $n = 50$ (Fig. 5f) and within 11.5% at $n = 20$.

Individual Assignment Tests

At the individual level, we could assign more geese to subspecies and management population, with greater confidence, using the combined microsatellite + mtDNA dataset, than using microsatellites alone, in both baseline (leave-one-out) and blind tests. Overall, the combined dataset allowed accurate assignments to subspecies; however, a proportion of individuals remained unassigned using the posterior probability threshold of >0.95.

Using the leave-one-out method and microsatellites alone, we were able to assign only 88 individuals (9.1%) to subspecies, and 94.3% of those were assigned to the correct subspecies. At the management population level, only 22 individuals (2.2%) were assigned, and 81.8% of those were assigned to the correct management population. Using the combined microsatellite + mtDNA dataset, we were able to assign 563 individuals (58.5%) to subspecies, and 97.5% of those were assigned to the correct subspecies. A larger proportion of giants (70.4%) could be assigned than interiors (34.2%; $\chi^2_1 = 117.6$; $P < 0.001$). At the management population level, 436 individuals (45.3% of total samples) could be assigned, including 388 MFGP

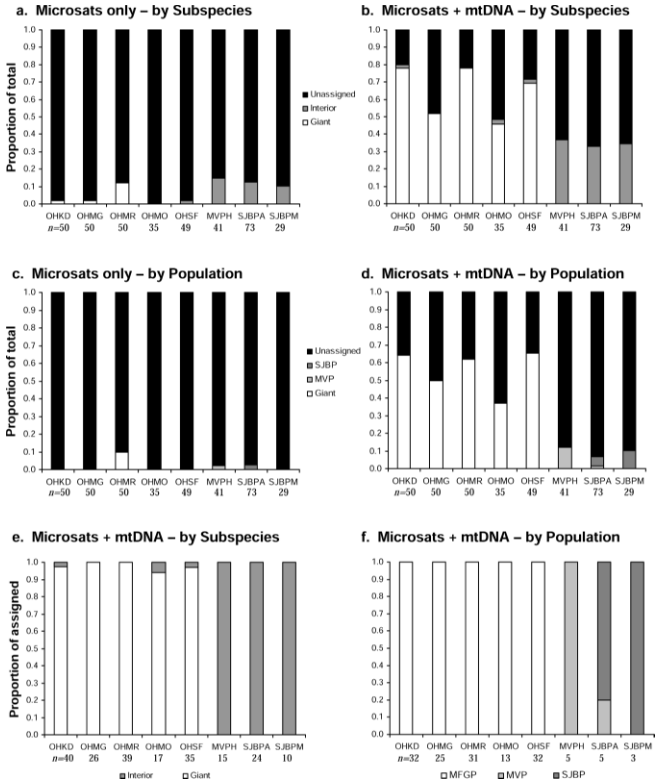

Figure 6. Individual assignment test results. We collected Canada goose samples of known origin (in the United States and Canada) during 2005, including 5 Ohio giant subpopulations (OHKD, OHMG, OHMR, OHMO, and OHSF) and 3 interior subpopulations (MVPH, SJBPA, and SJBPM) to test the ability of the assignment test to assign individuals correctly to subspecies (interior or giant) and management population (Mississippi Flyway Giant Population [MFGP], Southern James Bay Population [SJBP] or Mississippi Valley Population [MVP]). We determined the proportion of individuals assigned to subspecies using a) microsatellites, and b) microsatellites + mitochondrial DNA (mtDNA); the proportion of individuals assigned to management population using c) microsatellites, and d) microsatellites + mtDNA; and the proportion of all assigned individuals using microsatellites + mtDNA assigned to e) subspecies and f) management population. Refer to Table 1 and Figure 1 for subpopulation names and locations.

(60.5% of all MFGP samples), 29 MVP (17.6% of all MVP samples), and 19 SJBP (12.1% of all SJBP samples) individuals. Of those that could be assigned, 98% were assigned to the correct management population.

Using microsatellites alone, most individuals could not be assigned to subspecies or management population; only 0–14.6% of individuals from each subpopulation could be assigned to subspecies (Fig. 6a) and 0–10% to management population (Fig. 6c). Using the combined microsatellite + mtDNA dataset, 32.9–80% of individuals could be assigned to subspecies (Fig. 6b) and 6.8–64% to management population (Fig. 6d). A greater proportion of giant individuals could be assigned than interior individuals, at both the subspecies ($\chi^2_1 = 37.9$; $P < 0.001$) and management population level ($\chi^2_1 = 84.631$; $P < 0.001$); 67.1% of giants were assigned to subspecies and 56.8% to management population, whereas only 34.3% of interiors could be assigned to subspecies and 9.1% to management population. Those individuals that could be assigned were assigned with a high degree of accuracy: 94.1–100% of individuals for each blind subpopulation were assigned to the correct subspecies, with 98.1% of all giants and 100% of all interiors assigned correctly (Fig. 6e). At the management population level, 80–100% were assigned correctly, with 100% of MFGP, 100% of MVP, and 87.5% of SJBP assigned correctly (Fig. 6f). One SJBP individual was incorrectly assigned to the MVP population.

Harvest Estimates

The 3 genetic datasets yielded harvest composition estimates that varied by as much as 15.3% for the same harvest mixture. Results from simulations and blind tests suggest that the combined microsatellite + mtDNA dataset provided the most accurate and precise maximum likelihood estimates, so we assume estimates based on both sets of markers best represented true harvest composition. Differences between datasets were especially pronounced during the early season. Using the combined dataset, we estimated interiors accounted for 4.9% (95% CI = 1.7–8.0%) of the statewide early season harvest (Fig. 7e). Interiors accounted for an estimated 12.8% (95% CI = 4.9–20.8%) of the harvest using microsatellites alone (Fig. 7a) and 9.9% (95% CI = 4.7–15.0%) of the harvest using only mtDNA. This discrepancy was greatest in the South Zone, where the combined dataset suggested 8.5% (95% CI = 0.6–16.3) of the harvest was derived from interior populations, whereas microsatellites alone suggested 23.7% (95% CI = 6.0–41.5%). During the regular and late seasons, the discrepancy between datasets was not as pronounced; however, both microsatellites and mtDNA alone overestimated interior contributions (Fig. 7b, d). The combined dataset yielded a statewide interior estimate of 9.3% (95% CI = 6.9–11.6).

DISCUSSION

Our results suggest that 6 microsatellite loci do not allow accurate estimation of interior and giant Canada goose contributions to Ohio harvests and may give erroneous estimates of harvest derivations, despite the fact that previous studies in other geographic areas have successfully accomplished this using a similar set of microsatellite loci (Inman et al. 2003, Scribner et al. 2003, Shorey et al. 2007). This discrepancy suggests that a technique developed in one portion of a species' range may not be applicable to other geographic areas, due to genetic differences among populations. Maximum likelihood harvest derivations were highly dependent on the choice of genetic markers, emphasizing the importance of selecting appropriate markers that exhibit sufficient variation and have been shown through simulations and empirical testing to accurately discriminate among the subspecies or management populations of interest. We found that a combined microsatellite and mtDNA control region dataset allowed for accurate maximum likelihood estimation of proportional harvest contributions and we applied this technique to harvested samples.

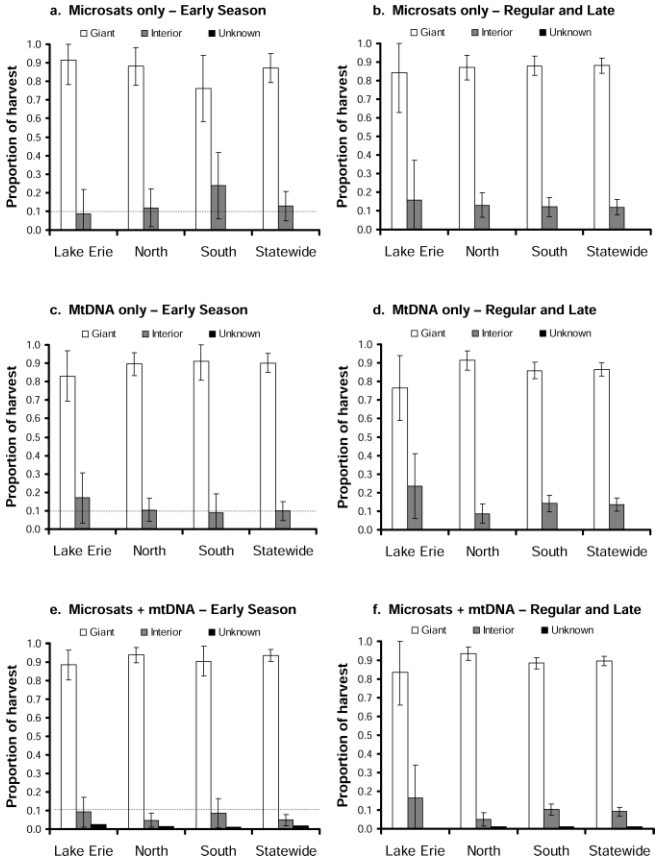

Figure 7. Maximum likelihood estimates of Canada goose harvest composition during Ohio's (USA) 2003–2004 season, including early season estimates of interior and giant proportions based on a) 6 microsatellite loci, c) mitochondrial DNA (mtDNA) control region sequences, and e) the combined microsatellite + mtDNA dataset; and regular and late season estimates based on b) microsatellites, d) mtDNA, and f) the combined microsatellite + mtDNA dataset.

Individual Versus Population-Level Estimates

Population-level mixed stock analysis was more successful than individual-level assignment tests at estimating harvest composition. Using individual assignment tests, a large proportion of birds could not be assigned to any source with high confidence; however, >95% of those that could be assigned were assigned to the correct subspecies and >85% to the correct management population when we included both microsatellites and mtDNA. A larger proportion of giants could be assigned than interiors, which could result in biased estimates of harvest composition. Assignment tests may be advantageous in cases where it is important to know the source identity of individual birds, but we suggest that population-level methods are more applicable for estimating harvest derivations.

Microsatellites Versus mtDNA

We found greater genetic differentiation between giant and interior subspecies in mtDNA control region sequences than in 6 microsatellite loci. The greater degree of divergence in mtDNA sequences is not unexpected, because mtDNA is maternally inherited and has an effective population size that is 25% that of nuclear markers (Avise 2004). Microsatellite homoplasy, allele similarity due to convergent or parallel evolution, may also lead to reduced variation, and high levels of within-population heterozygosity among highly variable microsatellite loci may reduce measures of differentiation (Hedrick 1999). Other possible explanations include divergence time and patterns of recent gene flow. The breeding range of interiors has been occupied only recently, as this area was covered in ice from the Pleistocene glaciations until <10,000 years ago (King and Martini 1984). Although this is sufficient time for some mtDNA differentiation to occur (Leafloor 1998), it may not have been long enough for substantial structure to occur in the nuclear genome. Reduced gene flow in maternally inherited mtDNA also may contribute to the magnitude of these differences. Both sexes exhibit strongly philopatric behavior, but females may exhibit slightly greater natal philopatry than males (Rohwer and Anderson 1988, Anderson et al. 1992, Leafloor 1998, Mowbray et al. 2002).

The success of MSA depends on adequate sampling of all contributing baseline populations, the number and degree of genetic differentiation among baseline populations, and the number and polymorphic nature of genetic markers used (Pella and Milner 1987, Epifanio et al. 1995, Kalinowski 2004). Our results support those of Pearce et al. (2000) in demonstrating the highly discriminatory nature of mtDNA, but neither microsatellites nor mtDNA alone were sufficient for discriminating among populations. The combined microsatellite + mtDNA dataset provided accurate and precise harvest derivations. Microsatellites alone have been used to discriminate among giant and interior populations in other parts of the Mississippi Flyway (Inman et al. 2003, Scribner et al. 2003); however, microsatellites yielded erroneous estimates of the composition of both simulated and known populations and overestimated harvest derivations of harvested samples in our Ohio study. These problems may be specific to the populations included in this study, and likely result from the genetic similarity, based on microsatellite markers, among some Ohio giant (OHMO and OHMG) and MVP subpopulations. We recommend using both sets of loci for estimating Canada goose harvest derivations, but in some cases the number of microsatellite loci may be reduced (Pearce et al. 2000).

Overall, the combined dataset provided accurate harvest derivations at the subspecies level, but estimates were less reliable for distinguishing between interior management populations. These difficulties likely arise from the lack of genetic structure between SJBPM and MVP populations. Banding studies suggest that long-distance natal dispersal sometimes occurs along the mainland coasts of Hudson and James bays, with several individuals banded as goslings on the Hudson Bay coast later recovered on James Bay and vice versa (Leafloor 1998). Therefore, the artificial boundary between the mainland SJBP and MVP breeding ranges may not accurately reflect biological boundaries between these populations, making it difficult to distinguish between them even with highly discriminatory genetic markers.

2003–2004 Harvest Composition

Harvest derivations, based on the combined microsatellite + mtDNA dataset, suggest that interior Canada geese comprised about 5% of the statewide early season harvest, well below the threshold of 10% required by the USFWS to allow a special early season (Federal Register 1995). The early season is intended to target temperate-nesting populations, while protecting subarctic-nesting migrants, and was first implemented in Ohio in 1994. Initial evaluation, based on banding, collar observations, and morphology, suggested that interiors did comprise <10% of the harvest (Tori and Shieldcastle 1995), and our results support these conclusions. Early seasons appear to be a minor source of mortality for subarctic-nesting populations (Tori and Shieldcastle 1995, Scribner et al. 2003, Sheaffer et al. 2005, this study); however, some harvest does occur in September, and monitoring of early seasons should continue to ensure that the impact on these populations remains minimal (Sheaffer et al. 2005).

During the 2003–2004 regular season, interiors accounted for about 9% of the combined regular and late-season harvest. Though based on only one season and small sample sizes, particularly in the Lake Erie Zone, our results suggest that interior harvest during the regular and late season was slightly higher in the Lake Erie Zone (16.7%) than in the other 2 zones (5% in the North Zone and 10% in the South Zone). Timing of migration and harvest of subarctic-nesting populations can vary significantly among years (Scribner et al. 2000, Shorey et al. 2007); therefore, it is important to examine multiple seasons, as well as spatial variation within seasons. Results of an ongoing multi-year study will better elucidate the spatial and temporal variation in the composition of Ohio's Canada goose harvest.

MANAGEMENT IMPLICATIONS

Genetic analysis provides an alternative to traditional harvest derivation methods (Scribner et al. 2003); however, our results suggest that maximum likelihood harvest derivations are highly dependent on the choice of genetic markers and that genetic methods should be rigorously tested and adjusted as necessary subspecies or management populations of interest. In some cases, where individual identity is important, assignment tests may be advantageous, but a significant portion of birds may not be assignable to any source population with confidence. Mixed stock analysis, using both microsatellites and mtDNA, will be valuable for determining spatial and temporal variation in Ohio Canada goose harvest composition, and the high discriminatory power of the combined microsatellite + mtDNA approach suggests that it will likely be applicable to widespread geographic regions.

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