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CONCORDANCE BETWEEN MORPHOLOGICAL AND MOLECULAR MARKERS IN ASSESSING HYBRIDIZATION BETWEEN SHARP-TAILED SPARROWS IN NEW ENGLAND

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Abstract.—Hybridization is pivotal in framing ideas about species concepts and has the potential to produce novel genotypes that may serve as starting points for new evolutionary trajectories. Presently, Nelson's Sharp-tailed Sparrows (*Ammodramus nelsoni subvirgatus*) and Saltmarsh Sharp-tailed Sparrows (*A. caudacutus caudacutus*) are in contact in salt marshes of Maine, New Hampshire, and northern Massachusetts. These two species hybridize, but the extent and direction of introgression has not been determined. We assessed morphological and genetic variation of 123 sharp-tailed sparrows from 5 salt marshes in New England. We used six morphological variables, including a plumage-scoring index, and five microsatellite primers to assess the extent of introgression and to determine whether there was concordance between phenotypic and genotypic variation. We identified apparent hybrids and each of the two sharp-tailed sparrow species using a plumage-scoring index. In general, we found that hybrids were more similar morphologically and genetically to Saltmarsh Sharp-tailed Sparrows. The alleles of hybrids were 62% Saltmarsh and 38% Nelson's Sharp-tailed Sparrows, supporting the asymmetrical hybridization hypothesis. *Received 18 July 2003, accepted 25 August 2004.*

Key words: *Ammodramus*, hybridization, introgression, microsatellite, morphological, salt marsh, sharp-tailed sparrows.

Concordancia entre Marcadores Morfológicos y Moleculares al Evaluar la Hibridación entre *Ammodramus nelsoni subvirgatus* y *A. caudacutus caudacutus* en Nueva Inglaterra

Resumen.—La hibridación es un proceso clave para plantear ideas sobre conceptos de especie y tiene el potencial de producir genotipos novedosos que pueden representar puntos de partida para nuevas trayectorias evolutivas. En la actualidad, los gorriones *Ammodramus nelsoni subvirgatus* y *A. caudacutus caudacutus* están en contacto en los pantanos de agua salada de Maine, New Hampshire y el norte de Massachusetts. Estas dos especies hibridan entre sí, pero el grado y la dirección de la introgresión no han sido determinados. En este estudio examinamos la variación morfológica y genética de 123 individuos de cinco pantanos ubicados en Nueva

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Inglaterra. Empleamos seis variables morfológicas, incluyendo un índice para calificar el plumaje y cinco iniciadores de microsatélites para determinar el grado de introgresión y determinar si existe concordancia entre la variación fenotípica y la variación genética. Identificamos híbridos aparentes e individuos de cada una de las dos especies usando un índice para calificar el plumaje. En general, encontramos que los híbridos fueron más similares morfológica y genéticamente a *A. caudacutus caudacutus.* El 62% de los alelos de los híbridos fueron de *A. caudacutus caudacutus* y el 38% de *A. nelsoni subvirgatus*, lo que apoya la hipótesis de hibridación asimétrica.

Hybridization, defined as the interbreeding of recognized species, presents challenges to the reconstruction of phylogenies, formulation of species concepts, and biological conservation (Grant and Grant 1992). It is also pivotal in framing ideas about taxonomic relationships (Short 1963) and understanding biological processes of evolution (Mayr 1963, Grant 1986). Genetic exchanges through hybridization have the potential to produce novel genotypes and phenotypes that may serve as starting points for new evolutionary trajectories (Lewontin and Birch 1966, Grant and Grant 1992, Arnold 1997), as well as expunge once distinct lineages (Gill 1997). Interest in avian hybridization has been rekindled in the past decade because ongoing transformations of natural landscapes have increased the likelihood of population expansions, contacts, and hybridization (Gill 1998).

During the breeding season, sharp-tailed sparrows (*Ammodramus* spp.) inhabit wet meadows, marshes, and salt marshes of central and eastern North America. The taxonomy, distribution, and evolutionary history of the group have been debated for more than a century (Dwight 1896, Montagna 1942, Beecher 1955, Greenlaw 1993, Rising and Avise 1993). In 1995, on the basis of morphological and genetic evidence, the American Ornithologists' Union (AOU) separated what had been considered a single species (*Ammodramus caudacutus*) with five known subspecies into two species: a northern species, Nelson's Sharp-tailed Sparrow (*A. nelsoni*, with three subspecies *A. n. nelsoni, A. n. alterus*, and *A. n. subvirgatus*) and a southern species, Saltmarsh Sharp-tailed Sparrow (*A. caudacutus*, with two subspecies *A. c. caudacutus* and *A. c. diverus*), limited to coastal wetlands (AOU 1995). *Ammodramus nelsoni subvirgatus* (hereafter "Nelson's sparrow") and *A. c. caudacutus* (hereafter "saltmarsh sparrow") are sympatric in coastal Maine, New Hampshire, and the northeast shore of Massachusetts (Hodgman et al. 2002) and have long been thought to hybridize (Montagna 1942, Rising and Avise 1993). Montagna (1940) collected a series of specimens from Popham Beach, Maine, that had intermediate characteristics between Nelson's and saltmarsh sparrows and considered that location an area of intergradation between those taxa. Furthermore, Rising and Avise (1993) found that 40% of assayed specimens from southern Maine were discordant between mitochondrial DNA (mtDNA) and morphology and suggested that there was some genetic exchange through hybridization.

Determining the extent of hybridization between Nelson's and saltmarsh sparrows is of particular interest because the latter are saltmarsh obligates with a limited breeding range. Consequently, that species has been identified as a high conservation priority on several ranking schemes (e.g. Partners in Flight, The Nature Conservancy; Carter et al. 2000). Knowing the extent of introgression between saltmarsh and Nelson's sparrows can enable (1) an understanding of the range limits for "pure" populations of both species, (2) determination of the direction of introgression, and ultimately (3) identification of the subspecies with the most pressing conservation needs. Our objectives here were to determine, through analysis of morphological and molecular markers, the extent of hybridization between Nelson's and saltmarsh sparrows in coastal New England.

Methods

We sampled sharp-tailed sparrows from five marshes along the New England coast (Table 1). We sampled two allopatric populations, one for each species, which were separated by 500 km and were ≥150 km from the closest sympatric population (Hodgman et al. 2002). We sampled Nelson's sparrows from Lubec, Maine, and saltmarsh sparrows from Prudence Island, Rhode

TABLE 1. Summary of plumage characteristics and scores used to generate a plumage index for Nelson's and Saltmarsh sharp-tailed sparrows breeding in coastal New England. Individuals with low scores were more typical of Nelson's Sharp-tailed Sparrow plumage characteristics, and individuals with high scores in a given category were more typical of Saltmarsh Sharp-tailed Sparrows.

Character description	Score
Breast and flank streaking; definition and amount (four categories)	
Very gray and washed-out, obscure streaks. No white mantle on feather. Very lightly streaked.	1
Gray and washed-out streaks with some chestnut color. Lightly streaked.	2
Mostly chestnut but washed-out and streaked.	3
Clear, dark chestnut streaking. Heavily streaked.	4
Clear, dark chestnut streaking with white mantle on feather. Densely streaked and uniform.	5
Whisker line definition and thickness (two categories)	
Very thick, gray and diffuse. Not clearly defined from the throat. Washed into throat plumage.	1
Thick, gray and diffuse but differing in color from throat.	2
Thin, gray and diffuse and defined from a white throat.	3
Thin, chestnut and clearly defined from a white throat.	4
Very thin, dark chestnut, and clearly defined from a white throat.	5
Face color and definition (two categories)	
Supercilium, fore-supercilium, auriculars, and malar washed-out orange or yellow. Fore-supercilium lighter or more yellow than supercilium and auriculars. Supercilium and auriculars not clearly separated by dark chestnut eye-stripe.	1
Supercilium, fore-supercilium, auriculars, and malar washed-out orange or yellow but more similar to each other. Not clearly defined from a chestnut triangular face patch that separates the supercilium from the auriculars.	2
Supercilium, fore-supercilium, auriculars, and malar orange and all having the same hue.	3
Supercilium, fore-supercilium, auriculars, and malar bright orange and clearly defined from a gray check patch and chestnut eye-stripe.	4
Supercilium, fore-supercilium, auriculars, and malar bright orange, clearly defined from a clean white throat by a thin whisker strip and from a gray check patch.	5
Back color and streaking (two categories)	
Back gray, not different from nape color. No streaking.	1
Back gray with small amount of chestnut. Faint streaking in two lines created by white mantle feathers.	2
Back gray-chestnut, differing from olive nape color. Two faint lines of white streaking.	3
Back chestnut, clearly different from olive nape color. Two lines of streaking on the outer sides of the back.	4
Back chestnut-brown with clear, distinct white streaking on outer side of back.	5
Median crown stripe width and definition (two categories)	
Crown stripe gray, 5 mm wide, and clearly defined by dark brown or chestnut lateral crown stripe.	1
Crown stripe gray, <5 mm wide, and slightly blended with lateral stripes.	2
Crown strip brownish-gray, <5 mm wide, and not clearly defined with lateral stripes.	3
Crown stripe olive-chestnut, thin, and difficult to distinguish from lateral stripes.	4
Crown stripe chestnut, almost identical in color to lateral stripes and very thin.	5
Bill color (one category)	
Entire lower mandible and bottom of upper mandible cerulean blue.	1
Entire lower mandible cerulean blue.	2
Greater than 50% of the lower mandible cerulean blue.	3
Less than 50% of the lower mandible faded blue, bottom of upper mandible yellow.	4
Lower mandible and bottom of upper mandible yellow.	5

Island, and considered them representative of each "pure" species that had been sampled as far away from any potential zone of overlap between the two species. We based that assumption primarily on the fact that saltmarsh sparrows were not detected on 30 surveyed marshes north of the northernmost sympatric population, and Nelson's sparrows were not detected on 165 surveyed marshes south of the southernmost sympatric population (Hodgman et al. 2002). We also sampled sparrows from three marshes within the zone of overlap (Table 1). We considered individuals that were sampled from marshes where both species occurred as sympatric Nelson's sparrows, sympatric saltmarsh sparrows, or hybrids. We examined differences in genetics and morphology among the geographic locations of the sympatric populations of both species. If no differences were detected in genetic or morphological variables among the three geographic locations, we maintained the sympatric groupings for further analysis. We developed a plumage index to determine levels of phenotypic introgression using study skins (Cornell University Museum of Vertebrates), field guides, and graphic depictions that described plumage differences between the two species (Sibley 1996). Plumage characters were divided into 13 equally weighted categories, summed to generate a plumage index (Table 1). A score of 1 was given for a category that represented a Nelson's sparrow, and a score of 5 was given for a category that represented a saltmarsh sparrow character. The plumage index could therefore range from 13, for a bird with all Nelson's sparrow plumage characters, to 65 for a bird with all saltmarsh sparrow plumage characters (Table 1). We defined hybrid sparrows *a posteriori* on the basis of the distribution of the plumage index score, such that the range of the plumage scores for hybrids did not overlap with the plumage scores from allopatric populations. The same observer (W.G.S.) scored plumage for all sparrows in the study.

We used mist nets to capture sharp-tailed sparrows from five marshes during the breeding season (31 May to 1 August 1998 and 1999) and attached metal bands and three color-bands to each individual. To assess morphological variation, we used digital calipers to measure bill length, depth, and width—all from the distal end of the nares (Grant 1986). We used a wing-chord ruler to measure wing chord and dividers to measure tarsus length. We weighed all sparrows to the nearest 0.25 g, using a spring scale.

We collected blood samples from the measured sparrows to extract DNA and match the phenotype and genotype of specific individuals. To obtain DNA, we drew 10–50 μL of blood from the brachial vein of sparrows from each group (Table 2) and stored the blood in a lysis buffer (0.1 mol Tris-Hcl, 0.01 mol EDTA). We extracted DNA from blood samples and standardized DNA concentrations following methods given in Yezerinac et al. (1995). We followed the methods of Dawson et al. (1997) for polymerase chain reaction (PCR) amplification and sizing of alleles at microsatellite loci. Amplification products radiolabeled with ^{33}P were resolved on 6% polyacrylamide denaturing gels containing 7.7 mol urea. Gels were run at 55 W for 2–3 h. When possible, products were double loaded. Radioactivity on dried gels was estimated using a Geiger-counter. Dried gels were exposed to BIOMAX (Dupont, Wilmington, Delaware) x-ray film. Exposure varied from one to seven days, depending on amount of radioactivity in the dried gel. Product sizes were determined by reference to a clone of a known size for each locus run in at least four lanes per gel (Dawson et al. 1997).

We used MANOVA and Tukey's *post hoc* tests to examine differences in the seven morphological variables (Zar 1999). We used principal components analysis (PCA) to visualize the relationships between (1) the morphological variables measured and (2) microsatellite alleles from the five loci examined (Roques et al. 2001). In both PCAs, we maintained all factors with eigen values >1 (Reyment et al. 1984). We plotted the mean factor scores (± 1 SE) for each population on the first two factors.

To determine levels of intrapopulation genetic diversity based on allelic composition, we used GENETIC DATA ANALYSIS, version 1.0 (Lewis and Zaykin 2001). We estimated population differentiation using F_{st}, as calculated by Weir and Cockerham (1984) in GENETIX (Belkhir et al. 1999); R_{st} (Goodman 1997); and Nei's D (Nei 1987). We used hierarchical cluster analysis to generate a Euclidean distance matrix for morphological variables (Manly 1986). We used Nei's D (Nei 1987) to generate a genetic distance matrix based on microsatellite allelic diversity. We compared those two matrices with

TABLE 2. Summary statistics for morphological variables (mean ± SE) measured on Nelson's and Saltmarsh sharp-tailed sparrows from five salt marshes in New England, 1998–1999. Lubec, Maine, represents the allopatric Nelson's Sharp-tailed Sparrow population and Prudence Island, Rhode Island, represents the allopatric population for Saltmarsh Sharp-tailed Sparrow. No differences in morphology were detected among the sympatric populations of either species.

	Lubec	Weskeag		Scarborough			Webhannet	Prudence Island
	Nelson's	Nelson's	Saltmarsh	Nelson's	Hybrid	Saltmarsh	Saltmarsh	Saltmarsh
Number of individuals	16	13	7	16	18	20	15	18
Bill length (mm)	8.58 ± 0.46	8.44 ± 0.37	9.57 ± 0.48	9.05 ± 0.41	9.66 ± 0.90	9.59 ± 0.65	9.61 ± 0.36	9.65 ± 0.32
Bill depth (mm)	3.86 ± 0.27	3.63 ± 0.68	3.79 ± 0.47	3.98 ± 0.38	3.96 ± 0.28	3.68 ± 0.40	3.77 ± 0.50	4.11 ± 0.30
Bill width (mm)	4.54 ± 0.30	4.75 ± 0.15	4.83 ± 0.09	4.28 ± 0.25	4.42 ± 0.35	4.82 ± 0.30	4.81 ± 0.10	4.80 ± 0.09
Tarsus length (mm)	21.02 ± 0.80	20.23 ± 1.72	19.79 ± 1.61	21.00 ± 0.57	20.61 ± 1.19	19.54 ± 0.80	20.47 ± 0.86	20.69 ± 0.66
Weight (g)	17.79 ± 1.09	19.42 ± 1.65	21.43 ± 0.67	18.19 ± 1.27	19.92 ± 2.02	20.46 ± 1.69	21.02 ± 1.28	19.97 ± 1.49
Plumage index	15.88 ± 2.63	20.69 ± 6.07	54.29 ± 6.10	21.50 ± 3.54	41.22 ± 7.07	54.65 ± 6.74	51.75 ± 7.87	60.72 ± 3.08
Wing chord (mm)	57.20 ± 1.42	58.15 ± 2.66	59.00 ± 2.69	57.67 ± 1.57	58.93 ± 1.71	56.95 ± 2.24	58.27 ± 2.38	57.56 ± 1.60

a Mantel test (Mantel 1967) to determine level of association between matrices and (based on 1,000 permutations) the probability of a test statistic greater than the calculated statistic by chance alone (Manly 1986).

We used ADMIX, version 2.0, to estimate the proportional contribution of the parental species to the genetic admixture of introgressed populations (Bertorelle and Excoffier 1998, Dupanloup and Bertorelle 2001). We used allopatric samples of both species as parental sources to quantify their allelic contributions (in percentages) to the sympatric groups. We used the bootstrap option, with 500 replications, to estimate allelic contributions and standard deviations. ADMIX 2.0 uses the mean coalescence time of genes within allopatric populations and between allopatric and sympatric populations to estimate admixture coefficients (Bertorelle and Excoffier 1998). Generally, those admixture coefficients are less biased than frequency-based estimators, especially when highly variable markers, like microsatellites, are used (Bertorelle and Excoffier 1998).

RESULTS

We captured, measured, and assessed genetic variation of 123 sparrows from five salt marshes in New England (Tables 2 and 3). The plumage index score ranged from 15.88 for Nelson's sparrows at Lubec, Maine, to 60.72 for saltmarsh sparrows at Prudence Island, Rhode Island (Table 2). Hybrids were defined as individuals with a plumage index score >31 and <55 (Table 2). We did not detect a difference ($P > 0.05$) in any morphologic variables or genetic distance measures among the sympatric populations for either species and, therefore, maintained our *a priori* groupings of sympatric populations (Table 3). The MANOVA results indicated an overall difference in morphological variables among the five groups (Pillia's trace = 1.164, F = 6.743, df = 28, $P < 0.001$; Wilks $\lambda = 0.156$, $F = 9.753$, df = 28, $P < 0.001$). All morphological variables, except wing chord, differed between at least two groups (Table 4). Hybrids were intermediate in four of six morphological measures (Fig. 1). Bill length was shorter for Nelson's sparrows than for saltmarsh sparrows ($P < 0.001$; Fig. 1). Bill depth differed only between allopatric saltmarsh sparrows and sympatric saltmarsh sparrows ($P = 0.003$). Bills of all Nelson's sparrows

TABLE 3. Sample sizes (n), geographic origins, number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and estimated boot-strapped proportions of Nelson's and Saltmarsh sharp-tailed sparrows allelic composition within each group.

Group	n	Geographic locations	Latitude and longitude	A	H_o	H_e	Nelson's (%)	Saltmarsh (%)
Allopatric Nelson's	16	Lubec, Maine	44°49.27′N 66°59.27′W	27	0.725	0.638	100.00	0.00
Sympatric Nelson's	29	Weskeag, Maine	44°04.60′N 69°08.66′W	40	0.643	0.643	76.09 ± 8.00	23.91 ± 8.00
		Scarborough, Maine	43°33.90′N 70°21.64′W					
Hybrids	18	Scarborough, Maine	43°33.90′N 70°21.64′W	42	0.811	0.739	37.67 ± 9.00	62.33 ± 9.00
Sympatric saltmarsh	42	Weskeag, Maine	44°04.60′N 69°08.66′W	41	0.705	0.665	20.43 ± 6.00	79.57 ± 6.00
		Scarborough, Maine	43°33.90′N 70°21.64′W					
		Webhannet, Maine	43°16.47′N 70°35.27′W					
Allopatric saltmarsh	18	Prudence Island, Rhode Island	41°37.49′N 71°19.43′W	36	0.744	0.680	0.00	100.00

TABLE 4. Results of MANOVA for seven morphological variables among the five populations of sharp-tailed sparrows. All variables differed between at least two populations, except wing chord.

Variable	Sum of squares	df	*F*	*P*
Bill length	20.740	4	11.134	<0.001
Bill depth	2.888	4	4.224	0.003
Bill width	3.335	4	11.727	<0.001
Tarsus length	21.833	4	5.018	0.001
Weight	127.639	4	31.910	<0.001
Wing chord	32.987	4	1.959	0.105
Plumage score	2,9957.228	4	73.192	<0.001

and hybrids were narrower than saltmarsh sparrows' bills ($P < 0.01$), but did not differ from each other ($P > 0.05$; Fig. 1). Both allopatric and sympatric Nelson's sparrow populations were ≤2.9 g lighter than hybrids and saltmarsh sparrows ($P < 0.001$; Fig. 1). All populations, except allopatric and sympatric Nelson's sparrows, differed significantly in plumage score ($P < 0.01$; Fig. 1). Hybrids were intermediate in plumage score among the populations (Fig. 1).

Sixty-two alleles from five polymorphic primer pairs flanking microsatellite regions were observed among the five groups (Table 5). Two loci (D*p*μ16 and Maμ23) each had one common allele, whereas the other three loci had a more even distribution of alleles across all populations (Table 6). Four alleles were diagnostic (two for each species) and only detected in the allopatric and sympatric populations (Table 6). Fifteen private alleles were distributed across all loci and occurred at low frequencies. Loci Asμ15 and Asμ18 had the greatest number of alleles (16 each), and Asμ18 had the most private alleles (5; Table 6). Private alleles from allopatric populations of both species represented 47% of the total (7 of 15; Table 6). Two private alleles (D*p*μ16-146 and Asμ18-138) were detected in hybrid individuals. We detected high genetic variability with unbiased heterozygosity values, ranging from 0.643 to 0.811 (mean = 0.726; Table 3). Weir and Cockerham's estimator of population subdivision (F_{st}) ranged from 0.014 to 0.143, R_{st} ranged from 0.029 to 0.263, and Nei's *D* ranged from 0.073 to 0.434 (Table 7). Allopatric Nelson's sparrows and allopatric saltmarsh sparrows were the most genetically different (Table 7). Three measures of genetic distance were significantly different between those populations (Table 7). Hybrids were most closely related to sympatric and allopatric saltmarsh sparrows (Table 7).

Using both morphological and molecular markers, we found similar patterns of relationship among the five groups, and there was a high level of association between those data (Mantel test = 0.979, $P = 0.02$). Thus, patterns of introgression evident at the phenotypic level were also expressed at the genotypic level. Principal components analysis indicated concordance between morphological and genetic markers among the five groups and identified hybrids as intermediate in both analyses (Fig. 2). The first morphological factor accounted for 35% of the variation and separated the five populations into distinct groups. Allopatric and sympatric Nelson's sparrows were clustered on the negative end of the first axis,

TABLE 5. Microsatellite primers used to assess the extent of hybridization between Saltmarsh and Nelson's sharp-tailed sparrows.

Name	Species	Number of alleles	Reference
D*p*μ16	Yellow Warbler	6	Dawson et al. 1997
Caμ02	Swainson's Thrush	18	Gibbs et al. 1999
Maμ23	Brown-headed Cowbird	6	Alderson et al. 1999
Asμ15	Grasshopper Sparrow	16	Bulgin et al. 2003
Asμ18	Grasshopper Sparrow	16	Bulgin et al. 2003

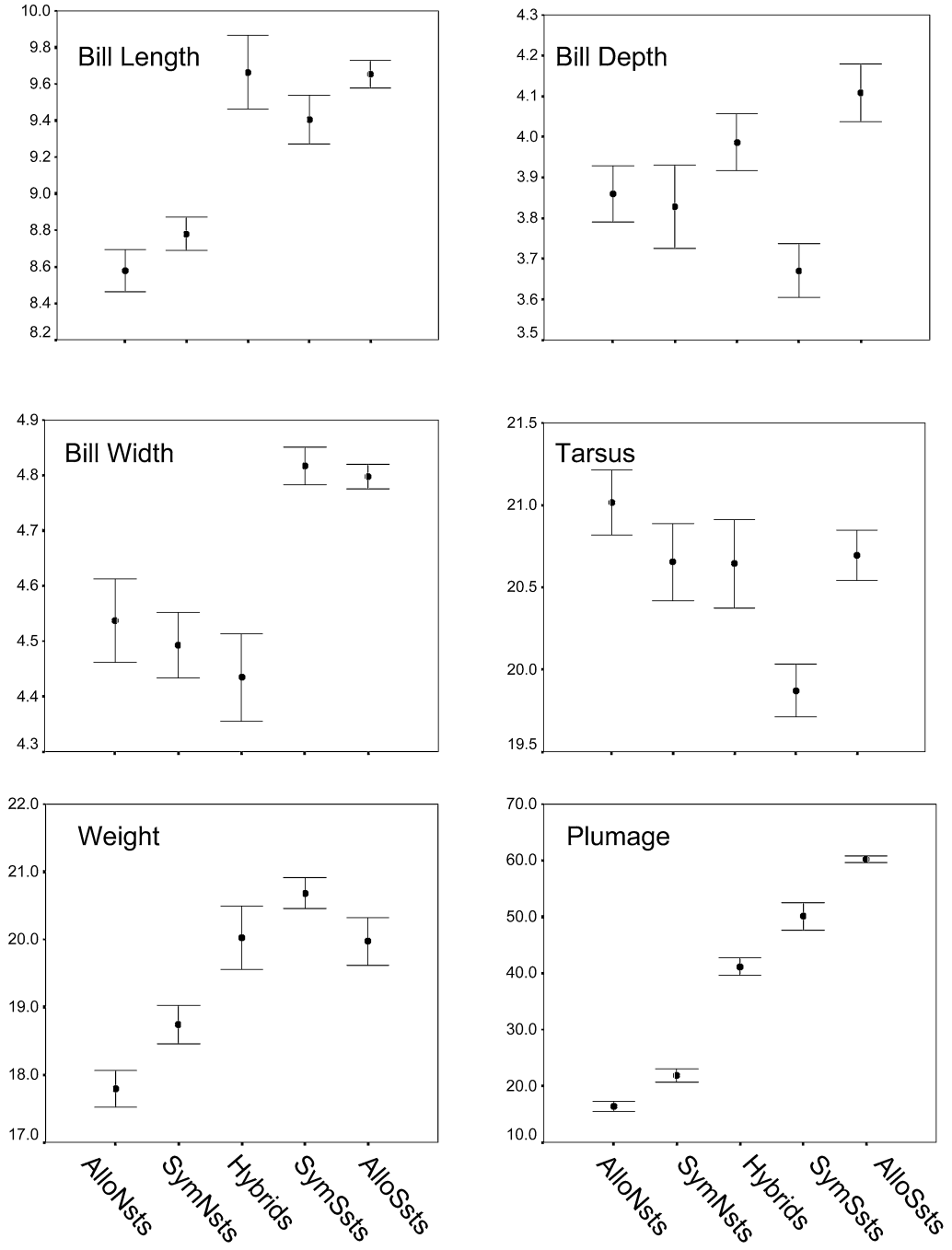

FIG. 1. Means (± SE) for six morphological variables that differed among the sharp-tailed sparrow populations. Hybrids were intermediate in bill depth (mm), tarsus length (mm), weight (g), and plumage score. Abbreviations: AlloNsts = allopatric Nelson's sparrow, SymNsts = sympatric Nelson's sparrow, SymSsts = sympatric saltmarsh sparrow, AlloSsts = allopatric saltmarsh sparrow.

TABLE 6. Allele frequencies in five populations of sharp-tailed sparrows from coastal New England. Four diagnostic alleles and 15 private alleles are shown in bold.

Locus	Allele	Allopatric Nelson's	Sympatric Neslon's	Hybrids	Sympatric saltmarsh	Allopatric saltmarsh
D*p*μ16	**136**	**0.13**	**0.14**	**0.07**	**0.00**	**0.00**
	138	0.00	0.00	0.00	0.00	**0.03**
	140	0.25	0.19	0.26	0.24	0.28
	142	0.63	0.67	0.64	0.73	0.67
	144	0.00	0.00	0.00	0.03	0.03
	146	0.00	0.00	**0.02**	0.00	0.00
Caμ02	**136**	0.00	0.00	0.00	0.00	**0.08**
	140	0.00	0.12	0.02	0.03	0.03
	142	0.00	0.31	0.07	0.00	0.00
	143	0.00	**0.02**	0.00	0.00	0.00
	144	0.53	0.33	0.05	0.03	0.08
	146	0.00	0.10	0.29	0.13	0.03
	149	0.00	**0.02**	0.00	0.00	0.00
	150	0.00	**0.02**	0.00	0.00	0.00
	152	0.03	0.00	0.05	0.08	0.08
	154	0.13	0.02	0.05	0.03	0.00
	156	0.00	0.03	0.02	0.27	0.28
	158	0.00	0.02	0.12	0.12	0.06
	160	**0.00**	**0.00**	**0.10**	**0.19**	**0.14**
	162	0.06	0.00	0.17	0.03	0.06
	164	0.00	0.02	0.00	0.08	0.06
	166	0.03	0.00	0.07	0.03	0.08
	170	0.06	0.00	0.00	0.00	0.03
	172	0.16	0.00	0.00	0.01	0.00
Maμ23	146	0.22	0.05	0.17	0.05	0.00
	148	0.00	0.05	0.05	0.05	0.03
	150	0.78	0.86	0.67	0.78	0.67
	152	0.00	0.00	0.10	0.06	0.00
	154	0.00	0.03	0.02	0.05	0.28
	160	0.00	0.00	0.00	0.00	0.03
Asμ15	**119**	0.00	**0.03**	0.00	0.00	0.00
	123	0.00	0.00	0.00	**0.03**	0.00
	125	0.09	0.03	0.07	0.01	0.00
	131	0.00	0.00	0.00	0.00	**0.03**
	133	0.16	0.16	0.00	0.00	0.08
	135	**0.44**	**0.38**	**0.07**	**0.00**	**0.00**
	137	0.22	0.10	0.07	0.06	0.03
	141	0.06	0.14	0.05	0.08	0.03
	143	0.00	0.05	0.24	0.18	0.11
	145	0.03	0.00	0.21	0.17	0.31
	147	**0.00**	**0.00**	**0.14**	**0.15**	**0.14**
	149	0.00	0.02	0.07	0.15	0.25
	151	0.00	0.05	0.02	0.12	0.00
	153	0.00	0.02	0.00	0.03	0.03
	155	0.00	0.00	0.00	0.01	0.00
	157	0.00	0.02	0.02	0.03	0.00
Asμ18	134	0.00	0.03	0.02	0.06	0.00
	136	0.00	0.03	0.02	0.06	0.00

Table 6. Continued.

Locus	Allele	Allopatric Nelson's	Sympatric Neslon's	Hybrids	Sympatric saltmarsh	Allopatric saltmarsh
Asμ18	**138**	0.00	0.00	**0.02**	0.00	0.00
	140	0.00	0.00	0.02	0.00	0.06
	142	0.00	0.00	0.00	0.00	**0.06**
	144	0.19	0.12	0.05	0.05	0.00
	146	0.25	0.26	0.10	0.06	0.03
	148	0.06	0.17	0.19	0.22	0.39
	150	0.06	0.12	0.29	0.35	0.39
	152	0.25	0.12	0.21	0.15	0.06
	154	0.09	0.05	0.05	0.03	0.03
	156	0.03	0.03	0.00	0.01	0.00
	158	0.00	0.03	0.02	0.00	0.00
	160	0.00	**0.02**	0.00	0.00	0.00
	162	**0.03**	0.00	0.00	0.00	0.00
	164	**0.03**	0.00	0.00	0.00	0.00

Table 7. Matrix of estimators of population subdivision, F_{st} (θ), R_{st} (ρ), and Nei's D values for five populations of sharp-tailed sparrows in New England.

		Sympatric Nelson's	Hybrids	Sympatric saltmarsh	Allopatric saltmarsh
Allopatric Nelson's	F_{st}	0.025	0.083[c]	0.119[c]	0.143[c]
	R_{st}	0.087[b]	0.062[c]	0.190[c]	0.212[c]
	Nei's D	0.089	0.264[c]	0.337[c]	0.434[c]
Sympatric Nelson's	F_{st}	–	0.041[c]	0.018[c]	0.110[c]
	R_{st}	–	0.101[c]	0.242[c]	0.263[c]
	Nei's D	–	0.187[c]	0.220[c]	0.316[c]
Hybrids	F_{st}	–	–	0.014	0.041[c]
	R_{st}	–	–	0.043[c]	0.056
	Nei's D	–	–	0.073	0.161[c]
Sympatric saltmarsh	F_{st}	–	–	–	0.018
	R_{st}	–	–	–	0.029
	Nei's D	–	–	–	0.076

Significance determined by 1,000 bootstraps for each estimator.
[a] Significant at $P < 0.05$.
[b] Signfican at $P < 0.01$.
[c] Significant at $P < 0.001$.

whereas both saltmarsh sparrow groups were clustered on the positive end of the first axis. Hybrids were positioned between the two species, but were more closely associated with saltmarsh sparrows. Phenograms constructed from distance matrices were also concordant between molecular and morphological markers and indicated that hybrids were more closely related to saltmarsh than to Nelson's sparrows (Fig. 3). Finally, using admixture analysis, we determined the genetic composition of hybrids to be 62.3 ± 9.0% saltmarsh sparrow alleles and 37.7 ± 9.0% Nelson's sparrow alleles (Table 3). Admixture analysis also revealed that sympatric groups of both species were not free from introgression. The allelic composition of sympatric groups was ~25% alleles from the other species (Table 3).

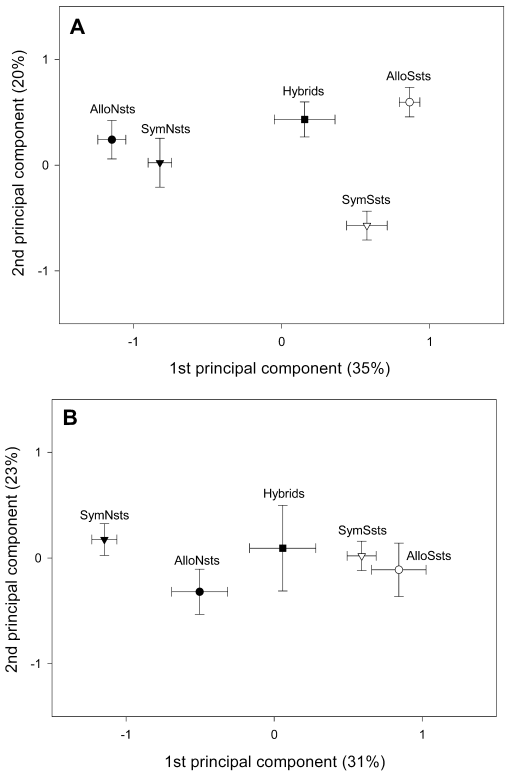

FIG. 2. (A) Mean morphological and (B) genetic factor scores (± SE) for each population. Hybrids were intermediate between pure populations for both data sets. Abbreviations same as Figure 1.

DISCUSSION

Morphological and microsatellite analyses revealed concordant patterns among the five groups of sharp-tailed sparrows breeding in New England. Allopatric Nelson's sparrows and allopatric saltmarsh sparrows differed most in both morphological and genetic measures. Nelson's sparrows had smaller bills, lower mass, and lower plumage scores than saltmarsh sparrows. There was no evidence of genetic exchange between allopatric populations, as shown by high levels of genetic divergence. However, we only sampled one allopatric population for each species, which may limit our interpretation of the extent of introgression, particularly in populations closer to the zone of overlap. We detected 15 private alleles, 7 of which were specific to allopatric populations, which supports the assumption that the allopatric populations were representative of their respective species. Allopatric and sympatric Nelson's sparrows were genetically similar, as were allopatric and sympatric saltmarsh sparrows. Hybrids were most genetically similar to sympatric saltmarsh sparrows on the basis of three genetic distance estimates and were intermediate between the allopatric groups in four of the six morphological measures. However, two bill-morphology metrics revealed potentially divergent patterns of introgression, with hybrids similar to saltmarsh sparrows in bill length and Nelson's sparrows in bill width.

Multivariate analyses of morphological data corroborated that sharp-tailed sparrows we defined as hybrids were more closely related to saltmarsh than to Nelson's sparrows. That pattern was confirmed by the genetic analysis, with ~50% greater proportion of saltmarsh sparrow alleles than of Nelson's sparrow alleles detected in hybrids. Estimated heterozygosity was highest in hybrid individuals, which is consistent with the concept that hybridization can act as a diversity-generating process (Seehausen 2004). Rising and Avise (1993), using mtDNA, estimated that 40% of the sharp-tailed sparrows sampled from one sympatric site were likely of hybrid origin and suggested that hybridization may be asymmetrical, with Nelson's females mating more randomly than saltmarsh females. Our results confirm that prediction and demonstrate that the direction of introgression likely occurs from saltmarsh to Nelson's sparrows, assuming that the allopatric sites sampled in our analysis were representative of the respective species. Rising and Avise (1993) stated that to confirm the direction of introgression, well-defined genetic markers from the nuclear genome would be required. The results of our analysis, which revealed that hybrids had 68% saltmarsh sparrow alleles and 32% Nelson's sparrow alleles, provide strong support for the Rising and Avise (1993) hypothesis that introgression may be asymmetrical. We also found that phenotypically "pure" individuals that occurred in sympatry had a relatively high proportion of their alleles from the nonparental species, potentially indicating a high level of backcrossing in sympatric populations.

Hybridization plays an important role in the evolution of plants and animals, and this evolutionary process creates major challenges for conservation biologists (O'Brien and Mayr

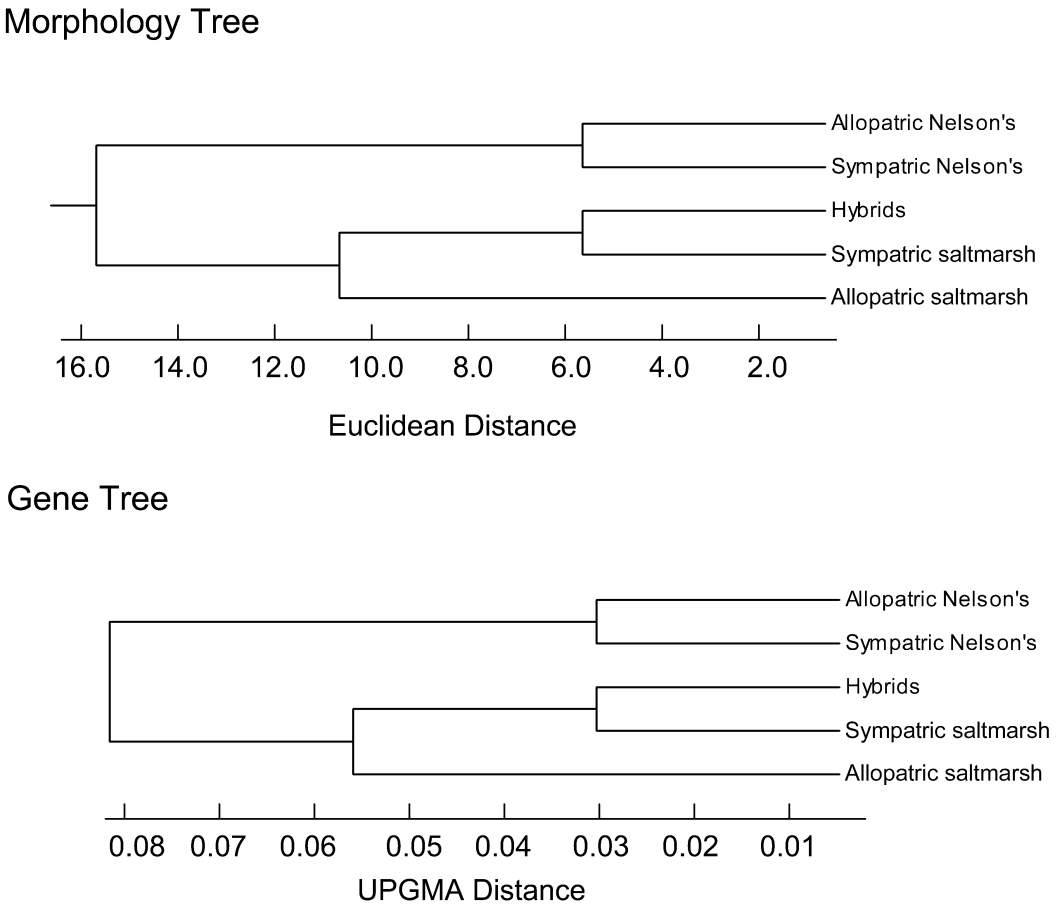

FIG. 3. Phenograms based on morphological and UPGMA (unweighted pair-group method with arithmetic means) distance matrices separating the five populations. Distance matrices were significantly positively correlated (Mantel test: $R = 0.979$, $P = 0.02$), which indicates a close association between morphologic and genetic data.

1991, Allendorf et al. 2001). Hybridization has contributed to the extinction of some species (Rhymer and Simberloff 1996), but has also played an important role in the maintenance and generation of genetic diversity (Grant and Grant 1992, Seehausen 2004). Categorizing hybridization to determine whether the situation is driven by anthropogenic or natural events can provide a framework for determining the necessary conservation actions and aid in setting priorities (Allendorf et al. 2001). Hybridization between sharp-tailed sparrows appears to be a natural introgression, where sympatric populations contain alleles from the other taxa, but more information may be necessary to determine whether the frequency of those alleles is changing over time.

Understanding the extent of hybridization has important conservation implications for sharp-tailed sparrows, especially the saltmarsh sparrow, which is limited to the narrow ribbon of coastal wetlands from Virginia to southern Maine (Greenlaw and Rising 1994). The results presented here indicate that gene flow between sympatric saltmarsh and Nelson's sparrows effectively limits the northern range of "pure" saltmarsh sparrows to northern Massachusetts (Hodgman et al. 2002). Because the two species are sympatric from Thomaston, Maine, to Parker River, Massachusetts, the northern limit

of "pure" saltmarsh sparrows is reduced by 190 km (~50 marshes), greatly increasing the conservation priority for this obligate salt-marsh breeding species in regions of allopatry.

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