

## ORIGINAL ARTICLE

# Amak Island Song Sparrows (*Melospiza melodia amaka*) are not evolutionarily significant

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## ORNITHOLOGICAL SCIENCE

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**Abstract** The conservation status and evolutionary distinctiveness of the isolated, small, and endemic population of Amak Island Song Sparrows (*Melospiza melodia amaka*) have been equivocal. Coupled with a reassessment of phenotypic evidence for this taxon, we used mitochondrial cytochrome b sequences and eight microsatellite loci to evaluate the relationship of the Amak population to nearby Song Sparrow populations. Phenotypically, *M. m. amaka* is not a valid taxon, and we found that Amak Song Sparrows possess no unique haplotypes and have allele frequencies and heterozygosity values similar to those in other populations. Congruence between genetic and morphological evidence suggesting no diagnosable differences leads us to propose that this population is not an evolutionarily significant unit (ESU), not a valid subspecies, not a distinct population segment (DPS), nor a diagnosable conservation unit, but rather a sink colonized by regional source populations.

**Key words** Cytochrome b, Evolutionarily significant unit, *Melospiza melodia amaka*, Microsatellite, Song Sparrow

Little is known about the taxonomy, demography, or conservation status of many morphologically-based subspecies found in remote or inaccessible locations, and endemic island taxa have proven particularly susceptible to extinction in historic times (BirdLife International 2000). Song Sparrows (*Melospiza melodia*) found on tiny Amak Island (55.4°N, 163.16°W), remote and difficult to reach in the Bering Sea (Fig. 1), represent such a problematic case. The putatively nonmigratory subspecies *M. m. amaka*, known only from Amak Island, was described based on the phenotype of six specimens (Gabrielson & Lincoln 1951). After examining the few specimens existing at that time, Gibson and Kessel (1997) tentatively submerged *M. m. amaka* in subspecies *M. m. sanaka*. A single individual from Amak was included in a genetic study (mitochondrial DNA restriction fragment length polymorphisms) of Alaska's Song Sparrows (Hare & Shields 1992). These genetic data were equivocal, in that this bird possessed a haplotype found in the Aleutian Islands (Fig. 1.)

Demographic information on the Song Sparrows of Amak Island is limited. Given the size of the island (10 km<sup>2</sup>) and limited survey data, the population is small, but some evidence suggests that it fluctuates markedly in size. Although survey details such as extent of island surveyed, weather conditions, and time of day and year are insufficient to verify the conclusions, the population was considered to be extinct in 1980 (Williams & Novak 1993), and anecdotal information indicated that 25 birds were seen in 1987 and four or five in 1988 (NatureServe 2003). This suggests that Amak Song Sparrows may have experienced severe population reductions (with near-extinction events) or that the Amak population may be in effect a sink, in that it might become extinct if not for immigration from nearby populations (Hanski & Simberloff 1997). Amak Song Sparrows are not listed as threatened or endangered by the U. S. Fish and Wildlife Service, but the Nature Conservancy considers this population to be imperiled (NatureServe 2003).

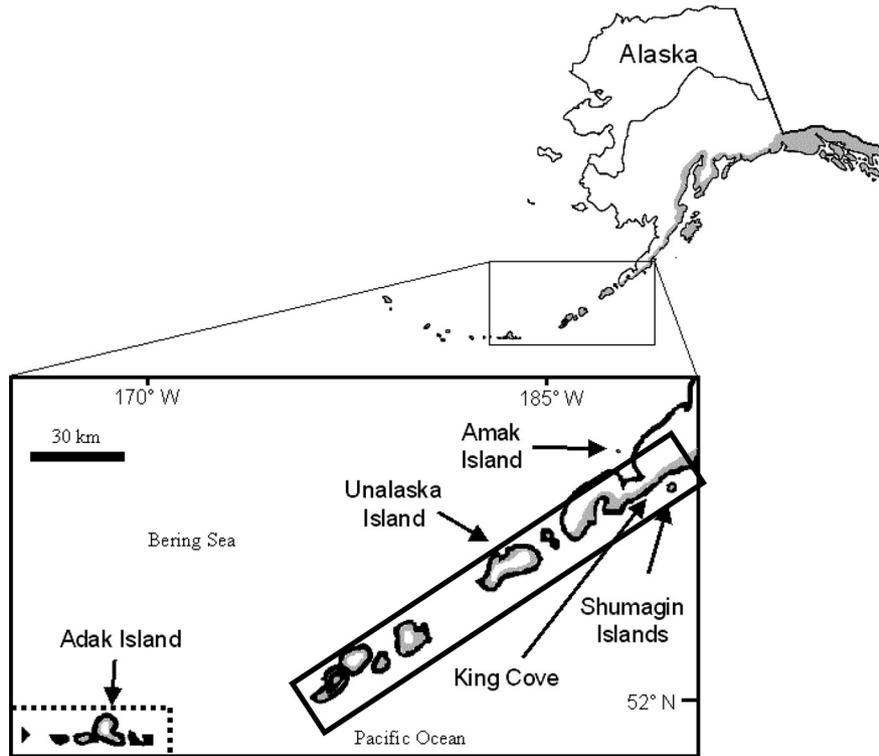
Resolution of the incongruous status of this population hinges on determination of the validity of the named taxon *M. m. amaka* and its evolutionary history. Because genetic data constitute primarily neutral variation and would be unlikely to include tiny por-

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**Fig. 1.** Map of Alaska with Song Sparrow (*Melospiza melodia*) collection locations used in this study. Alaska distribution of Song Sparrows shaded in gray. Boxes surround subspecies ranges with *M. m. maxima* outlined with a dashed black line and *M. m. sanaka* with a solid black line.

**Table 1.** Location, subspecies, number of individuals sequenced, number of individuals genotyped, expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosities and Genbank accessions for Song Sparrows (*Melospiza melodia*) used in this study. Museum voucher numbers provided in Appendix 1.

Location	Subspecies	Sequenced n	Genotyped n	$H_e$	$H_o$	GenBank accession
Amak Is., Bering Sea, Alaska	<i>amaka</i>	4	4	0.49	0.38	AY450608-611
King Cove, Alaska Peninsula, Alaska	<i>sanaka</i>	6	10	0.49	0.40	AY156406-411
Popof Is., Shumagin Is., Alaska	<i>sanaka</i>	4	9	0.56	0.49	AY156162-165
Unalaska Is., Aleutian Is., Alaska	<i>sanaka</i>	0	2	—	—	—
Adak Is., Aleutian Is., Alaska	<i>maxima</i>	10	30	0.42	0.40	AY156396-405

tions of the genome that might be under strong selection in peripheral populations, such data alone are not a reliable diagnostic tool for assessing subspecific validity (e.g., see Bulgin et al. 2003). Genetic data can provide invaluable insight into evolutionary history, however, and, when coupled with phenotypic assessments, congruent patterns between genotype and phenotype can be both insightful and diagnostic.

We reassessed phenotypic evidence and used mitochondrial (mt) DNA sequences and nuclear microsatellite loci to evaluate key questions about this little-known population: 1) Are Amak Song Sparrows

distinct from nearby populations?; and 2) Does this population show genetic evidence of severe reductions in size?

## MATERIALS AND METHODS

Whole genomic DNA from 55 Song Sparrows from Amak Island and four neighboring breeding populations (Fig. 1, Table 1) were extracted following Glenn (1997). Most of the mtDNA cytochrome b gene (1,137 bp) was amplified and cycle-sequenced using four primer pairs per individual for a subset of

the extracted tissues (Table 1). Primers used included: L14851 (Kornegay et al. 1993), H16064 (Harshman 1996), L15350 (Klicka & Zink 1997), and H15424 (Hackett 1996). Amplified products were sequenced in both directions using an ABI 373A or 3100 automated sequencer (Applied Biosystems Inc., Foster City, CA). All sequences were deposited in GenBank (Table 1). All birds used in this study were sampled during the breeding season, including the four individuals from Amak. All sampled populations are considered to be non-migratory (Murie 1959). Thus, it is likely that the birds examined represented the local breeding populations at these locations.

Eight microsatellite loci were amplified for all individuals using fluorescent dye-labeled primers developed for Song Sparrows (Jeffery et al. 2001) and for two other bird species (*Escu1*, Hanotte et al. 1994; *GF5*, Petren 1998). Amplicons were screened for variation using an ABI 373A or 3100 automated sequencer. Average expected and observed heterozygosities for each population (except Unalaska) were determined using GDA (Lewis & Zaykin 2001).

Phenotypic assessment was done using new material and traditional taxonomic methods of visual comparisons of external phenotype—the same methods used in the original assessment of Amak Island Song Sparrows (Gabrielson & Lincoln 1951) and known to be effective in other drab-plumaged passerines (e.g., Winker 1997). All known existing *M. m. amaka* specimens (N=9) and several hundred each of *M. m. sanaka* and *M. m. maxima* were included in these examinations.

## RESULTS

Even with new material, we did not find *M. m. amaka* phenotypically separable from *M. m. sanaka* or *M. m. maxima* using the classic “75% rule” (Amadon 1949; Patten & Unitt 2002). This is concordant with the reassessment of Gibson and Kessel (1997), in which the original material used by Gabrielson and Lincoln (1951) did not seem to support the original erection of a subspecies *amaka*. The assertion that Song Sparrows from tiny Amak Island are separable from nearby Song Sparrows at Unimak Island on the Alaska Peninsula and in the Shumagin Islands was founded on an alleged distinctness in several plumage and mensural characters in a type series of only six “adult” specimens (Gabrielson & Lincoln 1951:253). The plumage characters are equivocal: “Resembles *maxima* from the western Aleutians

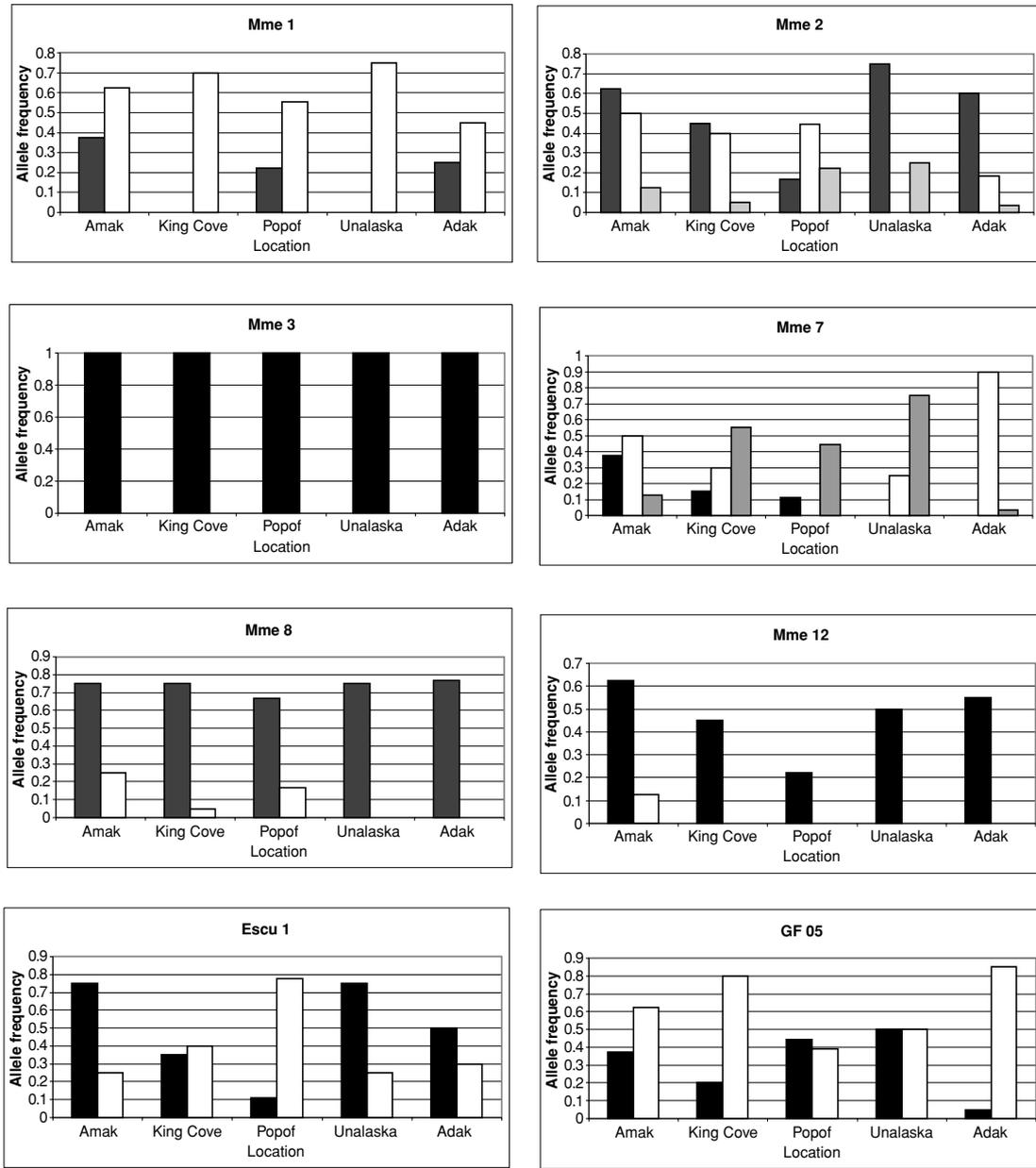
in color and extensive brown markings, but somewhat more heavily marked with brown than that race both on back and breast; in most available specimens the brown markings also somewhat brighter. Closer in color to *maxima* than to the geographically closer race *sanaka*.” And neither these plumage characters nor the average culmen measurement given for the four male and two female specimens will separate with certainty even one Amak specimen from a series of adjacent *sanaka*. This equivocal situation is not at all alleviated with the new material now in the University of Alaska Museum.

Only four of Gabrielson and Lincoln’s (1951) original six specimens are present today at the U. S. National Museum, and these include the adult male holotype, a second adult male, one immature male, and one immature female (fide R. C. Banks, in litt., 2004). Thus, the original plumage description conflates both sexes and two age classes in the description of six “adults.” We think the authors should have heeded their own caveat regarding problems separating *maxima* from *sanaka* in the central-eastern Aleutians (in their description of subspecies *maxima*—published in the same article as their description of “*amaka*”): “The series of specimens in similar plumage is too limited to make a certain decision” and “The tail and wing measurements vary somewhat more than normal on account of wear and are not fully reliable” (Gabrielson & Lincoln 1951: 251–252). With such small sample sizes statistical tests are not useful where there is considerable overlap in putatively diagnostic characters. Despite the fact that plumage characters can be well assessed visually, and although we do support the subspecies concept, we find nothing phenotypically to justify continued recognition of this subspecies.

Genetically, there are no unique cytochrome b haplotypes found on Amak Island. Haplotype A is shared with Adak Island, and haplotype B is found in high frequencies at King Cove and the Shumagin Islands (Table 2; Fig. 1). There is a single unique allele (locus *Mme 12*; Fig. 2) in the Amak samples; all other alleles are found at frequencies similar to those from other sampled locations (Fig. 2). Observed and expected heterozygosities are comparable to values in other populations (Table 1).

## DISCUSSION

Gibson and Kessel (1997) tentatively lumped Amak Island Song Sparrows (*M. m. amaka*) into the



**Fig. 2.** Frequencies of microsatellite alleles from eight loci among Amak Island Song Sparrows and the frequencies of these alleles in other populations. Each allele is shaded differently to track its frequencies in other populations.

**Table 2.** Frequency of mtDNA cytochrome b haplotypes at each collection location in Alaska.

Location	Haplotype		
	A	B	C
Amak Island, Bering Sea	1	3	0
King Cove, Alaska Peninsula	0	5	1
Popof Island, Shumagin Islands	0	4	0
Adak Island, Aleutian Islands	10	0	0

neighboring subspecies, *M. m. sanaka*, which is found on the Alaska Peninsula and the eastern Aleutian Islands. Our assessment, which includes substantial new material obtained since their evaluation, supports this treatment. We recognize that sample sizes of *amaka* are small. Because this is a small population in an isolated and inaccessible location large sample sizes were not possible for this study. Given logistical and ethical constraints, these samples are not likely to be substantially increased. Only 11 spec-

imens of “*amaka*” have been collected over the last 80 years (Gabrielson & Lincoln 1951; Hare & Shields 1992; this study). We deem our evidence sufficiently conclusive to warrant reporting now for management considerations of Amak sparrows.

Genetically, Amak Song Sparrows share haplotypes with populations from two nonmigratory subspecies (*M. m. maxima* from Adak Island and *M. m. sanaka* from our other sample locations) that have no haplotypes in common. This suggests that Amak Song Sparrows might be intergrades or that this population was colonized by individuals from both subspecies. Interestingly, Gabrielson and Lincoln’s (1951) original description of *M. m. amaka* included phenotypic evidence of such intergradation.

In the Aleutian Islands and on the Pacific coast of the Alaska Peninsula, Song Sparrows are found among rocky beaches and beachside grasses (Gabrielson & Lincoln 1951, Murie 1959). Thus, Song Sparrows can only inhabit a narrow ring around Amak Island. Even if optimal conditions existed around the entire perimeter of the island (which they do not; KW pers. obs.), this population would always be small. Considering these limitations together with anecdotal suggestions of fluctuations in population size, it is likely that Amak Song Sparrows would exhibit very little genetic diversity if this population were evolutionarily isolated. Although only four individuals were sampled, heterozygosities were similar to those in other populations. This suggests that immigration to Amak from other populations is ongoing. A similar pattern was found for Song Sparrows on Mandarte Island, British Columbia (Keller et al. 2001), in which very low numbers of migrants caused a rapid recovery in a genetically bottlenecked population.

Amak Island is a small volcano that appeared above the sea about 6,700 years ago (Marsh & Leitz 1979). It is visible from the mainland coast, only 17 km away. Volcanic activity was reported in the 1700s but had ended by 1867 (Dall 1870). Thus, Amak Song Sparrows probably colonized within the last several thousand years, and subsequently they may have experienced severe ecological disturbances. Coupled with the harsh climatic conditions currently found in this region, cycles of extinction and recolonization have probably characterized this population since its founding.

The overarching question about using small sample sizes is whether larger sample sizes would alter the conclusions of the study. Phenotypically the an-

swer to this is no; new material does not bear out Gabrielson and Lincoln’s (1951) suggestion that *amaka* is a diagnosable subspecies. Nor does their original material suggest (phenotypically) that a formerly endemic population has gone extinct and been replaced by new colonists. This is in contrast to the one other subspecies that Gabrielson and Lincoln (1951) described at the same time; *maxima* is a valid subspecies (Gibson & Kessel 1997, unpubl. data).

Genetically, the four Song Sparrows from Amak shared haplotypes with adjacent populations. If more individuals were examined from Amak and were found to have other haplotypes, this would not diminish the importance of the four sparrows that possessed haplotypes found in other populations. In addition, increasing microsatellite sample sizes would likely increase the heterozygosity levels found on Amak. However, given the probable demographic shifts and the current heterozygosity values for the Amak population, it seems improbable that we would have sampled the only sparrows that were genetically diverse. Thus, gene flow would be inferred regardless of sample size.

Overall, our evidence suggests that “*amaka*” was a weak subspecies that does not hold up under scrutiny. Based on this evidence, we suggest that these birds are simply a peripheral extension of other, larger regional Song Sparrow populations. Recognition of Amak Song Sparrows as an evolutionarily significant unit (ESU), a distinct population segment (DPS), or a management unit (MU) is not warranted.

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**Appendix.** Voucher numbers for specimens used in this study.

Subspecies	Museum <sup>a</sup>	Catalog numbers	Locality
<i>Melospiza melodia amaka</i>	UAM	13425–13428	Alaska: Bering Sea, Amak Island
<i>Melospiza melodia sanaka</i>	UAM	9321, 9328, 10091, 11230, 11362, 11365–66, 11381, 11389, 11823	Alaska: Alaska Peninsula, King Cove
<i>Melospiza melodia sanaka</i>	UAM	10090, 10171, 10187, 11276, 11379, 11390, 11585, 11713, 12142	Alaska: Shumagin Islands, Popof Island
<i>Melospiza melodia sanaka</i>	UAM	111238–239	Alaska: Aleutian Islands, Unalaska Island
<i>Melospiza melodia maxima</i>	UAM	8460–61, 10040–42, 10167–68, 10170, 10172, 10179, 10188, 10942, 10946–47, 11048, 11175–78, 11267–69, 11501, 11511, 11827, 11850, 12143, 13057, 13059, 13161	Alaska: Aleutian Islands, Adak Island

<sup>a</sup> UAM=University of Alaska Museum.