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17. Artificial diets were prepared by adding 5.0 g of finely ground plant material to 50 cm^3 of a 1% agar solution. For the catkin plus leaf diet, 2.5 g of each was added. For the catkin plus tannin treatment, 0.1 g of quebracho extract (from the tree *Aspidosperma quebracho*) was added to the catkin and agar mixture. Quebracho extract is a complex mixture of condensed tannins and flavonoids. These mixtures were poured onto trays and spread to a thickness of about 2 mm and stored in a refrigerator at 6°C. Fresh 1 by 1 cm squares were provided to the caterpillars each day.
18. For caterpillars raised at 25°C and 14 hours of light, 3 out of 28 (10.7%) raised on catkin died before pupation, whereas 10 out of 29 (34.5%) raised on leaf diet died before pupation. These proportions are thus not the same (z approximation, $z = 2.25$, $P < 0.02$).
19. Bird exclosure experiments on oak trees in the Chiricahua Mountains showed that predation by birds can reduce the biomass of foliage-dwelling arthropods by about 50% in some years [E. Greene, thesis, Princeton University (1989)]. Approximately 60% of wild-caught caterpillars from *Q. arizonica* and *Q. emoryi* were parasitized, mainly by ichneumonid and braconid wasps and tachinid flies.
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Molecular Genetic Relationships of the Extinct Dusky Seaside Sparrow

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Mitochondrial DNA from the extinct dusky seaside sparrow (*Ammodramus maritimus nigrescens*) was compared in terms of nucleotide sequence divergence to mitochondrial DNAs from extant populations of seaside sparrows. Analyses of restriction sites revealed a close phylogenetic affinity of *A. m. nigrescens* to other sparrow populations along the Atlantic coast of the United States but considerable genetic distance from Gulf coast birds. Concerns and applied management strategies for the seaside sparrow have been based on a morphological taxonomy that does not adequately reflect evolutionary relationships within the complex.

THE DUSKY SEASIDE SPARROW, A melanistic form (*1*) of *Ammodramus* sparrow discovered in 1872 (*2*), had a native range confined to Brevard County, Florida (*3*). During the 1960s, a population formerly numbering in the thousands was in severe decline, largely because of artificial flooding of marsh-grass habitat for mosquito control and conversion of land to pasturage (*4*). In 1966, the dusky was listed as "endangered" by the U.S. Fish and Wildlife Service (*5*). By 1980, only six birds (all males) could be found (*6*). Five of these were brought into captivity in a last-ditch effort to preserve genes of the subspecies. In a program of captive breeding with Scott's seaside sparrow (*A. m. peninsulae*) (*7*), first-generation female hybrids between dusky males and Scott's females were crossed to dusky males, yielding backcross progeny with an expected preponderance of dusky nuclear genes (Fig. 1). Several hybrids ranging from 50 to 87.5% dusky have been produced (*8*); they constitute the core population for a contemplated reintroduction of dusky-like birds into the wild (*7*).

The last pure dusky seaside sparrow died on 16 June 1987. Here we compare mitochondrial DNA (mtDNA) isolated from its tissues with that of other geographic and taxonomic populations in the seaside sparrow complex. The intent is to critically evaluate the evolutionary genetic history of *A. m. nigrescens*, the taxonomic entity toward which government and private management efforts have been directed.

We chose to analyze mtDNA for several reasons. First, mtDNA in vertebrates evolves very rapidly at the nucleotide sequence level (*9*), and hence provides high resolution for distinguishing recently separated populations such as those within a species (*10*). The problem of genetic distinction is especially acute for lower taxa of birds, where genetic differences are typically small (*11*). Second, because mitochondria are maternally inherited in vertebrates (*12*), mtDNA can be used to identify the matriarchal ancestry of individual animals, without the complications of allelic segregation and

Table 1. Clonal descriptions and subspecies distributions of mtDNA genotypes observed in seaside sparrows. Letters in the descriptions, from left to right, refer to multifragment mtDNA profiles produced by digestion with Ava I, Ava II, Bam HI, Bcl I, Bgl I, Bgl II, Cla I, Eco RI, Hin cII, Hin dIII, Msp I, Nde I, Pst I, Pvu II, Spe I, Sst II, Stu I, and Xba I. Adjacent letters in the alphabet differ by a single restriction site, nonadjacent letters by at least two restriction site changes. Numbers in parentheses are references.

Clone	mtDNA description	Traditional subspecies designation	No. of birds
1	C C C C C C C C C C C C C C C C C C	*maritima, macgillivraii* (*14*), *nigrescens*	17
2	C C C C C C C C C C C C C C B C C C	*macgillivraii* (*14*)	1
3	C C C C C C C C B C C C C C C C C C	*maritima*	1
4	C C C C C C C D C C C C C C C C C C	*macgillivraii*	1
5	C B C C C C C C C C C C C C C C C C	*maritima*	1
6	C G D C C C C D C C D C C G C C C C	*fisheri, juncicola, peninsulae*	14
7	C G D C C C C D C C E C C G C C C C	*fisheri*	1
8	C G D C C C C D C C F C C G C C C C	*peninsulae*	1
9	C H D C C C C D C C D C C G C C C C	*peninsulae*	1
10	C G D C C C C D C B D C C G C C C C	*fisheri*	1
11	C G D C C C B D C C D C C G C C C C	*juncicola*	1
		Total	40

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recombination that apply to nuclear genes. Finally, because of this mode of transmission, mtDNA from the last male duskies has not been transmitted to hybrids in the restorative breeding program (Fig. 1). Thus, unlike the case for genes in the cell nucleus, the last obtainable in vivo mtDNA from *A. m. nigrescens* expired with the death of the last dusky male.

We isolated mtDNA by CsCl gradient centrifugation (*13*) from fresh heart plus liver tissue of six [or seven (*14*)] of the nine recognized subspecies of *A. maritimus* (Fig. 2), as follows: *A. m. maritimus*, $n = 7$ individuals, Suffolk County, New York; *A. m. macgillivraii*, $n = 4$, New Hanover County, North Carolina, $n = 3$, Charleston County, South Carolina, $n = 2$, Duval County, Florida, $n = 4$, Nassau County, Florida; *A. m. nigrescens*, $n = 1$, Brevard County, Florida; *A. m. peninsulae*, $n = 5$, Levy County, Florida, $n = 2$, Dixie County, Florida; *A. m. juncicola*, $n = 6$, Wakulla County, Florida; and *A. m. fisheri*, $n = 6$, Cameron Parish, Louisiana. Mitochondrial DNA from each individual was digested by each of the 18 informative restriction endonucleases listed in Table 1. Fragments were radioactively end-labeled with one or more ^{35}S-labeled nucleotides, separated by molecular weight through 1.0 to 1.8% agarose gels, and visualized by autoradiography. Restriction site differences among digestion profiles were determined by comparisons of mtDNA fragment sizes against a 1-kb molecular weight ladder (Bethesda Research Labs). Estimates of nucleotide sequence divergence were made with the "fragment" and "site" approaches of Nei and Li (*15*). The resulting matrix of genetic distances was used to construct a phenogram by UPGMA clustering (*16*). Evolutionary networks were also constructed from a presence-absence matrix of restriction sites by Wagner and Dollo parsimony methods (*16*).

A total of 110 restriction sites was observed in the survey, with an average of 89 sites (representing 482 bp of recognition sequence) scored per individual. Eleven differentiable mtDNA genotypes (or clones) were represented in our sample of 40 seaside sparrows (Table 1). Many clones were very closely related, differing by only one or two restriction site gains or losses. However, clones belonging to two distinct arrays differed by six to nine restriction site changes and a mean sequence divergence of $P = 0.0109$. After correction for mtDNA polymorphism within each array (*15*), the net nucleotide divergence between groups remained $P = 0.0098$. One array included all individuals sampled from Atlantic coast populations; the other included all Gulf coast samples (Fig. 2). The fundamental

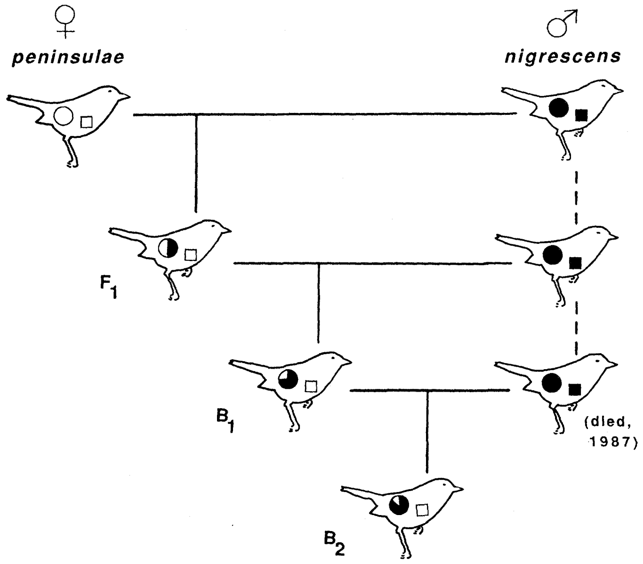

Fig. 1. A pedigree in the captive breeding program for the dusky seaside sparrow. Three *nigrescens* males, the last of which died in 1987, were involved. The darkened areas within circles and squares represent the expected proportions of nuclear and mitochondrial genes, respectively, of dusky origin.

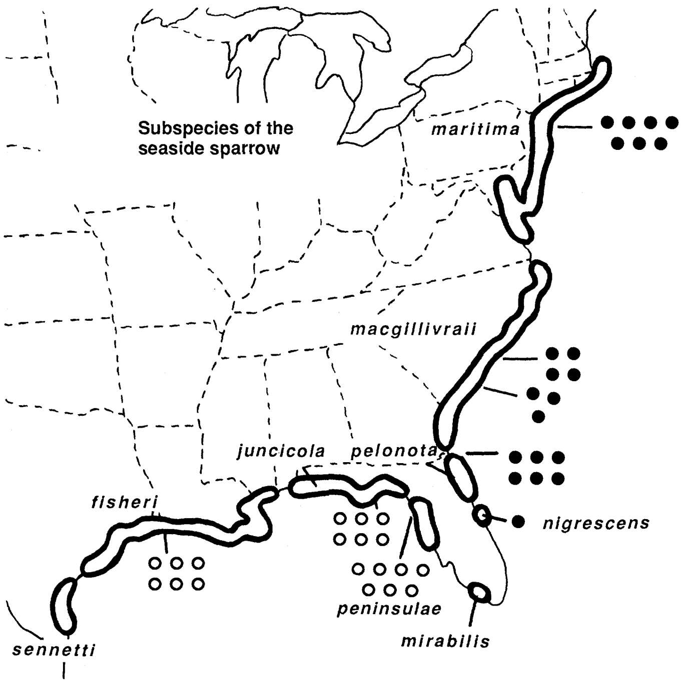

Fig. 2. Geographic distributions of the taxonomically recognized subspecies of the seaside sparrow. Open and closed circles represent birds exhibiting, respectively, the distinctive Gulf coast and Atlantic coast mtDNA genotypes observed in the present study (see Fig. 3 and text).

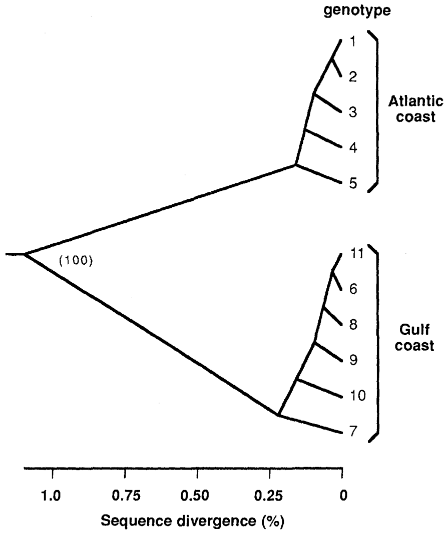

Fig. 3. UPGMA dendrogram showing the distinction between mtDNA genotypes of Atlantic coast versus Gulf coast populations of the seaside sparrow. In the Wagner networks generated by the PENNY analyses, many equally parsimonious networks were obtained, but all involved minor branch rearrangements within these Atlantic or Gulf clonal arrays. One of these Wagner networks (identical in structure to UPGMA) is shown here. The number in parentheses indicates that the phylogenetic separation of mtDNA in Atlantic and Gulf coast forms was supported at the 100% level among 200 computer bootstrapped samples (*16*). In the DOLLO parsimony analysis, the shortest length networks also cleanly distinguished all Atlantic from Gulf coast birds.

distinction in mtDNA genotype between Atlantic and Gulf sparrows was evident in both of the parsimony methods of analysis, as well as in the UPGMA summary (Fig. 3). Mitochondrial DNA from the dusky seaside sparrow was identical at all surveyed restriction sites to the most common clone in other Atlantic coast populations (Table 1 and Fig. 3).

The phylogenetic patterns for mtDNA of the seaside sparrow probably evidence a long-term separation of Atlantic and Gulf coast populations. If mtDNA in sparrows has evolved at the rate reported for mammals (*9*) and other birds (*17*), approximately 2 to 4% sequence divergence per million years, then these two populations may last have been in contact some 250,000 to 500,000 years ago. While this estimate of absolute age must remain qualified due to uncertainties about exact calibration of the mtDNA evolutionary clock (*10*), there can be little doubt that the relative ages of mtDNA separations between Atlantic and Gulf clones greatly exceed those within either region.

From a phylogenetic perspective, mtDNA represents a single nonrecombining "gene," and hence reflects the history of only a small fraction of a total organismal pedigree. Nonetheless, the mtDNA phylogeographic pattern receives additional support from an evolutionary hypothesis previously advanced by Funderburg and Quay (*18*). From a consideration of sparrow morphology, zoogeography, and the geologic history of the southeastern United States, they proposed a fundamental phylogenetic split distinguishing all Atlantic from all Gulf populations of *A. maritimus*. Seaside sparrows are relatively sedentary and restricted to salt marshes. Emergence of the Florida peninsula during the early Pleistocene was suggested as a likely factor responsible for severing gene flow among populations inhabiting a formerly continuous salt marsh habitat across what is now northern or central Florida (*18, 19*). Extensive red mangrove (*Rhizophora mangle*) forests presently provide a strong ecological barrier to seaside sparrow dispersal around the current southern tip of the Florida peninsula (*18*).

The mtDNA data provide no basis for phylogenetic distinction of the dusky seaside sparrow from other Atlantic coast populations of *A. maritimus*. By the criterion of mtDNA sequence, the last *A. m. nigrescens* appears to have been a routine example of the Atlantic coast phylad of seaside sparrow. Doubt has previously been raised about the evolutionary distinctiveness of the dusky seaside sparrow: from an overview of all named subspecies, Kale (*4*) concludes that the dusky "In all respects . . . is a typical Seaside Sparrow." Of course, no study can prove the null hypothesis that significant genetic differences between taxa are absent. Nonetheless, the burden of proof would now seem to rest more squarely on any proposal for a special evolutionary status for *A. m. nigrescens*.

If an intraspecific taxonomy for the seaside sparrow had reflected the fundamental phylogenetic distinction between Atlantic and Gulf coast populations, and the relative paucity of genetic differences among populations within either region, management programs for the species would likely have been very different from what they are now. First, in the absence of a formal species or subspecies designation for the "dusky seaside sparrow," exceptional preservation efforts mandated by the Endangered Species Act would not have applied to the Brevard County population (*20*). Second, a strategy to reestablish "native-like" birds into Brevard County would probably have involved release of hybrids between the dusky and other Atlantic rather than Gulf coast birds (*21*). Third, conservation efforts would have been directed toward insuring the viability and genetic integrity of the two major phylogenetic subunits within the species.

Taxonomy and systematics are sometimes considered among the least important or challenging of the biological disciplines [for counterarguments, see Wilson (*22*)]. However, taxonomic assignments inevitably shape our basic perceptions of the biological world, influencing choice of research topics and interpretation of results. In this study, we have provided evidence that a taxonomy for the seaside sparrow, which was initiated in the last century and upon which management decisions continue to be based, does not properly summarize the evolutionary genetic relationships of the populations involved. This is not the first instance in which a faulty taxonomy has resulted in well-intentioned but misdirected efforts in endangered species management (*23*). A widened concern with the phylogenetic bases for taxonomic decisions should contribute to the recognition and conservation of biotic diversity.

REFERENCES AND NOTES

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19. A marshy corridor along what are now the Suwanee and St. Marys river basins and the Okefenokee swamp may have existed at various times of high sea level since the Miocene (*18*). The extents and exact times of sea water inundation of the "Suwanee straits" area, and their effects on biotic distributions, are poorly known [see T. M. Bert, *Mar. Biol.* **93**, 157 (1986), and references therein]. A related possibility involves a continuous salt-marsh connection, at times of high sea level, around the tip of a truncated Florida peninsula [S. M. Stanley, *Palaios* **1**, 17 (1986)].
20. Emphatically, we do not wish to be interpreted as arguing against the desirability of preserving remaining natural populations and habitats; this should always remain a high priority.
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