EVOLUTION AND CONSERVATION OF TIDAL-MARSH VERTEBRATES: MOLECULAR APPROACHES

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Abstract. The tidal marshes of North America are home to a diverse collection of morphologically differentiated reptiles, birds, and mammals. We reviewed the existing molecular studies on endemic tidal-marsh vertebrates, including turtles, snakes, sparrows, rails, shrews, and rodents. We found both deep and shallow divergences from their nearest upland relatives in all geographic regions. In the Northeast, the Saltmarsh Sharp-tailed Sparrow (*Ammodramus caudacutus*) has probably been isolated from the inland forms of the Nelson's Sharp-tailed Sparrow (*A. nelsoni*), for >600,000 yr, while the salt-marsh form of the Swamp Sparrow (*Melospiza georgiana*) evolved from upland relatives <40,000 yr ago. On the West Coast, saltmarsh forms of the Song Sparrow (*M. melodia*) and the ornate shrew (*Sorex ornatus*) show low levels of genetic differentiation from neighboring upland forms, while the salt marsh harvest mouse (*Reithrodontomys raviventris*) living in the same marshes shows deep genetic divergences from upland forms dated to nearly 4,000,000 yr ago (MYA). On the Gulf Coast, saltmarsh Clapper Rails (*Rallus longirostris*) show either a very recent split from, or high levels of gene flow with freshwater King Rails (*R. elegans*), but Seaside Sparrows (*A. maritimus*) probably diverged from an upland ancestor 1.5–2 MYA and diamondback terrapins (*Malaclemys terrapin*) 7–11 MYA. The timing of those divergences ranges from late Miocene to late Holocene, and suggest a complex history of multiple invasions and differentiations in saltmarshes. Molecular approaches have increased our understanding of the evolutionary origin of these unique forms, revealed the complex patterns of genetic structure within them, and furthered conservation efforts.

Key Words: Adaptation, allozymes, *Ammodramus*, genetic structure, geographic variation, *Malaclemys*, *Melospiza*, microsatellites, mitochondrial DNA, morphology, *Nerodia*, *Rallus*, *Reithrodontomys*, *Sorex, tidal marsh*.

EVOLUCION Y CONSERVACION DE VERTEBRADOS DE MARISMA DE MAREA: ENFOQUES MOLECULARES

Resumen. Las marismas de marea de Norte América son el hogar de una diversa colección de reptiles, aves y mamíferos morfológicamente diferenciados. Revisamos los estudios moleculares existentes de vertebrados endémicos de marismas de marea, incluyendo tortugas, culebras, rascones, gorriones, musarañas y roedores. Encontramos divergencias tanto profundas como poco profundas de sus parientes más cercanos de las tierras más altas en todas las regiones geográficas. En el Noreste, el Gorrión Cola Aguda de marisma salada (*Ammodramus caudacutus*) ha sido probablemente aislado de las formas de las tierras interiores del Gorrión Cola Aguda Nelson (*A. nelsoni*), por mas de 600,000 años, mientras que la forma de marisma salada del Gorrión Pantanero (*Melospiza georgiana*) evolucionó de parientes de tierras más altas hace menos de 40,000 años. En la costa oeste, formas de marismas saladas del Gorrión Cantor (*M. melodia*) y de la musaraña vistosa (*Sorex ornatus*) muestran bajos niveles de diferenciación de formas de vecinos de las tierras mas altas, mientras que el ratón de cultivo de marisma salada (*Reithrodontomys raviventris*) viviendo en las mismas marismas, muestra profundas diferencias genéticas de formas de tierras mas altas, que datan de hace aproximadamente 4,000,000 años (HMA). En la Costa del Golfo, rascones de marisma salada (*Rallus longirostris*) muestran ya sea de una separación muy reciente, o elevados niveles de flujo genético con el Rascón Real (*R. elegans*) de agua fresca, pero los Gorriones Costeros (*A. maritimus*) probablemente divergieron de un ancestro de tierras mas altas 1.5–2 HMA y la tortuga acuática (*Malaclemys terrapin*) divergió hace 7–11 HMA de su forma ancestral. El tiempo en el que transcurrieron esas divergencias de rango del Mioceno tardío al Holoceno tardío, sugieren una historia compleja de invasiones múltiples y diferenciaciones en marismas saladas. Enfoques moleculares han aumentado nuestro entendimiento del origen evolutivo de estas formas únicas, revelando los patrones complejos de la estructura genética entre ellas, y han ayudado al progreso de esfuerzos de conservación.

Saltmarsh bird species, such as the Seaside Sparrow (*Ammodramus maritimus*), the Saltmarsh Sharp-tailed Sparrow (*A. caudacutus*) and the Clapper Rail (*Rallus longirostris*) have been known to science for >200 yr (American Ornithologists' Union 1983). Based on research in the past century, vertebrate zoologists have now described 25 species or morphologically differentiated subspecies of avian, mammalian and reptilian species largely or wholly restricted to tidal marshes (Hay 1908, Grinnell 1909, 1913; Clay 1938; Marshall 1948a, b; Boulenger 1989). Tidal marshes are discrete in their distribution and present a profound environmental disjunction, and therefore may

impose intense directional selective pressures on colonizing populations. Much speculation exists over the evolutionary history of these tidal-marsh forms and with the advent of molecular techniques many avenues for exploration have opened. Genetic markers have become important tools for evolutionary and conservation biologists. We can now use genetic markers to determine phylogenetic relationships, clarify the sister taxa of tidal-marsh endemics and to time their divergence from ancestral taxa. This enables the direct testing of evolutionary hypotheses relating to the origin of tidal-marsh endemics and the circumstances under which they diverged. A variety of molecular markers, such as allozymes, restriction fragment length polymorphisms (RFLPs), DNA sequences, and microsatellites have been applied to tidal-marsh taxa, providing estimates of divergence, gene flow, and population differentiation (Table 1).

For conservation biologists, the use of molecular techniques can provide genetic estimates of intraspecific variation important for evaluating the viability and adaptive potential of endangered populations (Smith and Wayne 1996). Molecular markers are also particularly important for determining the taxonomic status of endemic taxa, both for defining conservation units and for advocating appropriate management action. Improper lumping of distinct species can result in underestimates of regional biodiversity and splitting of non-distinct taxa can divert valuable resources and reduce opportunities for proper genetic management (Frankham et al. 2002). Furthermore, molecular techniques can verify hybrids that are suspected based on morphology (Frankham et al. 2002).

In addition to macroevolutionary and conservation questions, tidal-marsh taxa lend themselves to the study of microevolutionary

TABLE 1. A VARIETY OF DIFFERENT MOLECULAR MARKERS, SUCH AS ALLOZYMES, RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs), DNA SEQUENCES, AND MICROSATELLITES, HAVE BEEN EXAMINED IN TIDAL MARSH TAXA, PROVIDING ESTIMATES OF AMONG SPECIES AND AMONG POPULATION DIFFERENTIATION.

Molecular marker	Description
Allozymes	Protein allozyme variation is derived from alleles separated based on their charge as they migrate through a gel medium, most often, starch. Individuals are assigned a genotype, and heterozygosity and allele frequencies can be calculated. An important statistic, F_{st}, which is a measure of among population genetic variance, can be calculated from allele frequencies. F_{st} ranges from 0–1, with a value of zero indicating panmixia and lack of genetic differentiation, and a value of 1 indicating fixation of alternate alleles in each population and therefore a lack of gene flow. Estimates of F_{st} based on allozymes for avian taxa are extremely low (average F_{st} = 0.022, SD = 0.011), but are higher for mammalian taxa (average F_{st} = 0.230, SD = 0.183) (Barrowclough 1983).
RFLPs	Restriction enzymes are used to cleave DNA at specific recognition sites and then the DNA fragments are separated by weight on a gel. Often mitochondrial DNA is isolated and is used in order to reduce the number of fragments. Mutations within the recognition sites produce a variable banding pattern and an estimated percent nucleotide sequence divergence can be calculated. For avian taxa, interspecific divergence among congeners have ranged from 0.07%–8.8% (Avise and Lansman 1983).
DNA sequences	With the invention and widespread application of the polymerase chain reaction (PCR), researchers now have the ability to produce sequences affordably and from small amounts of tissue. DNA sequence data provides both phylogenetic information and information on population genetic structure based on allele frequencies. Percent sequence divergence can be used to estimate the time to the most recent common ancestor if the divergence between taxa using DNA sequences is calibrated from the fossil record. The use of mtDNA sequences has become very important for the field of phylogeography (Avise 2000).
Microsatellites	Microsatellites are tandem repeats of simple sequences that occur frequently and at random throughout the genome. These highly polymorphic markers are flanked by unique sequences that serve as ideal sites for the design of primers that can be used for PCR amplification. Since the microsatellite polymorphism is stable and is inherited in a Mendelian fashion these markers can be highly informative. PCR primers can be labeled with one of the four currently available fluorescent dyes. After PCR, the products are separated on acrylamide gels and using a scanning laser and commercially available software, primers labeled with different dyes alleles can be distinguished even when their sizes overlap. Allele sizes are reproducibly and accurately determined. Allele frequencies, heterozygosity, and F_{st} estimates can be calculated.

processes (Avise 2000). First, tidal saltmarsh habitat is discrete and relatively homogeneous simplifying the understanding of the spatial configuration underlying the genetic variation. Second, advances in our understanding of marsh history (Malamud-Roam et al., *this volume*) may allow biologists to estimate the timing of habitat availability for tidal marsh organisms, improving hypotheses for divergence times of upland and tidal marsh forms. Third, tidal-marsh forms are often found in close proximity to conspecific or closely related non-tidal marsh populations, yet face a different set of environmental challenges. The combination of these three factors provides a unique framework within which we can attempt to understand the role of selection on short temporal scales; and the sensitivity of selective forces to gene flow, with the advantage of multiple geographic replicates and with a diverse group of vertebrates that includes reptilian, avian, and mammalian taxa.

In this chapter we present an overview of studies in which molecular markers have been used to study tidal-marsh vertebrate taxa. Much evolutionary work has been devoted to certain groups, such as sparrows in the genera *Ammodramus* and *Melospiza*. Several other studies have focused on determining the taxonomic status and genetic basis for differentiation in endemic saltmarsh populations such as those found in the diamondback terrapins (*Malaclemys terrapin*), water snakes (*Nerodia fasciata* and *N. sipedon*), Clapper Rail (*Rallus longirostris*), Savannah Sparrow (*Passerculus sandwichensis*), shrews (*Sorex ornatus* and *S. vagrans*), and harvest mice (*Reithrodontomys raviventris* and *R. megalotis*). These case studies are followed by a synthesis of patterns that have emerged from the genetic study of tidal-marsh taxa and recommendations for new avenues of research.

A central issue addressed in these case studies is the timing of divergence between tidal-marsh and related non-tidal marsh taxa. Divergence times are based on molecular clock studies that calibrate rates of base pair substitutions using independent fossil or geological evidence. In many cases the studies reviewed here attempted to estimate a divergence date based on mitochondrial DNA (mtDNA) data. In most birds, for example, the cytochrome *b* gene has been found to have a substitution rate of 1–3% per million years (Fleischer and McIntosh 2001), and many authors use 2% per million years as a rough clock for estimating dates of cladogenesis (Klicka and Zink 1997, Avise and Walker 1998). A number of potential problems are associated with applying a molecular clock beyond the taxa for which it was estimated, including rate heterogeneity across lineages, calibration

error, and overestimation of divergence time due to ancestral polymorphism (Edwards and Beerli 2000, Arbogast et al. 2002). For example, the overestimation due to ancestral polymorphism for cytochrome *b* in birds is likely to be about 175,000 yr, or on average 12% of avian haplogroup divergence is taken up by ancestral polymorphism (Moore 1995). These problems still need to be addressed in many of these taxa, however, with these caveats in mind, patterns of divergence across tidal marsh taxa may still be comparable.

CASE STUDIES

DIAMONDBACK TERRAPIN

Systematics, distribution, and ecology

The North American diamondback terrapins are medium-sized emydids that exploit and are confined to brackish coastal waters on the eastern coast of the US, from New York state to Texas. Emydid turtles are normally characteristic of freshwater ecosystems in the Americas, Europe, North Africa, and Asia. Although a handful of species have colonized productive estuarine areas, most cannot survive in sea water (Davenport and Wong 1986; Davenport et al. 1992). Unique among emydid turtles, *Malaclemys* is physiologically capable of spending several weeks in sea water without frequent access to fresh water (Gilles-Baillen 1970, Dunson 1985) and has therefore attracted much physiological and ecological study (Robinson and Dunson 1975).

Malaclemys terrapins have a suite of behavioral, physiological and morphological traits that allow them to occupy euryhaline environments ranging in salinity from 11–31 ppt (Dunson 1985). Their wide geographical distribution, perhaps coupled with limited gene flow between brackish-water populations separated by open coast, has led to an unusual degree of recognized subspeciation. The genus is monotypic and seven subspecies have been recognized throughout its range. Pritchard (1979) describes the following subspecies (running from north to south in the species' distribution): *M. t. terrapin* (found from Cape Cod to Cape Hatteras), *M. t. centrata* (a subspecies overlapping with the northern subspecies and stretching to Florida), *M. t. tequesta* (Florida east coast terrapin), *M. t. rhizophora* (an obscure subspecies found in Florida mangroves), *M. t. macrospilota* (the ornate diamondback of the southern part of the Gulf Coast of Florida), *M. t. pileata* (the Mississippi diamondback, distributed to eastern Louisiana from the Florida Panhandle),

and finally the Texas diamondback, *M. t. littoralis*, which is distributed from Louisiana to Corpus Christi in southern Texas.

Phylogenetic analysis of morphological and molecular character data indicate map turtles (*Graptemys*) are closely related to *Malaclemys* and are likely a sister taxon (Gaffney and Meylan 1988, Bickham et al. 1996, Lamb and Osentoski 1997). Map turtles are largely riverine and with 12 species, the genus is the largest in the family Emydidae.

Evolutionary history and biogeography

A Pleistocene divergence between *Malaclemys* and *Graptemys* was proposed by Wood (1977) and was investigated by Lamb and Osentoski (1997) using molecular markers. Examination of the cytochrome *b* locus in mtDNA between *Malaclemys* and *Graptemys* revealed a deep divergence (1.54–3.11% sequence divergence). Assuming a cytochrome *b* evolutionary rate of 0.2–0.4% per million years in turtles (calibrated against fossil evidence and biogeographic barriers) Lamb and Osentoski (1997) estimated that *Malaclemys* and *Graptemys* may have diverged from a common ancestor some 7–11 MYA during the late Miocene and not during the Pleistocene. Thus the evolution of this group is the earliest example of divergence of a terrestrial tidal marsh vertebrate from its freshwater ancestor to date.

Genetic structure and within-species processes

Molecular markers have also been used to address genetic differentiation among populations of *Malaclemys* and within species evolutionary processes. Although cytochrome *b* sequence divergence values within *Malaclemys* subspecies range from 0.0–0.38% (Lamb and Ostentoski 1997), terrapins from the Atlantic assemblage north of Cape Canaveral were differentiated by restriction enzyme analysis as well as sequence analysis from a Gulf Coast assemblage from south Florida westward. This phylogeographic split detected within *Malaclemys* by Lamb and Osentoski (1997) supports prior inferences of the distinctness of Atlantic and Gulf Coast diamondback terrapins by Lamb and Avise (1992) who looked at mtDNA restriction site variation (N = 53 from Massachusetts to western Louisiana).

Lamb and Osentoski (1997) proposed that a regional vicariant event resulting from Pleistocene glacial maxima shaped the mtDNA divergence within *Malaclemys*. During Pleistocene glacial maxima, sea levels dropped approximately 150 m in the Gulf of Mexico,

exposing extensive portions of the west Florida shelf as well as portions of the Yucatan Peninsula (Poag 1973). This land-mass expansion, coupled with increased aridity in the southeast and hypersaline conditions at the mouth of the gulf, likely isolated the gulf's estuarine ecosystems from those in the Atlantic.

Despite the finding that mtDNA haplotypes differed between Atlantic and Gulf coast populations, microsatellite markers showed low overall genetic differentiation between terrapin populations from New York, North and South Carolina, the Florida Keys, and Texas (S. Hauswaldt, pers. comm.). Analysis of a total sample of 320 individuals at six microsatellite loci provided evidence that East Coast terrapins were more similar to Texas terrapins than either group was to the terrapins from the Florida Keys—a pattern the researchers attributed to the well-documented translocation of Texas terrapins to the Atlantic Coast after the early-20th-century depletion of terrapin stocks by over harvest (S. Hauswaldt, pers. comm.).

In summary, mtDNA variation has demonstrated an ancient divergence for diamondback terrapins from their closest freshwater relative, the map turtles. Furthermore, molecular markers have supported the hypothesis of a Pleistocene vicariant event isolating Atlantic and Gulf coast populations. Finally, anthropogenic translocation of terrapins has left a genetic signature detected using microsatellite loci.

WATER SNAKES

Systematics, distribution, and ecology

Among vertebrates that inhabit tidal marshes, members of the water snake complex (*Nerodia fasciata-sipedon-clarkii*) provide an example of divergence at several levels. As previously recognized, *N. fasciata* of the southeastern US was comprised of six subspecies that could be clearly divided into two groups on the basis of ecology and physiology. The freshwater group (*N. f. fasciata, N .f. confluens,* and *N. f. pictiventris*) occupies an extensive area of freshwater habitats along the coastal plain of eastern North America (Conant 1975). The salt-water group (*N. f. clarkii, N. f. compressicauda*, and *N. f. taeniata*) inhabits a narrow coastal saltwater zone and are distributed almost continuously from the mid-Atlantic Coast of Florida to southern Texas, including the Florida Keys (Conant 1975). Progressive loss of saltmarsh habitat on the east coast of Florida has resulted in *N. f. taeniata* being listed by USDI Fish and Wildlife Service as a threatened species.

The snakes in the salt-water group are physiologically well adapted to exploit their saline environment (Zug and Dunson 1979; Dunson 1980). The geographic ranges of the saltwater and freshwater groups sometimes overlap in the saltwater-freshwater ecotone, yet, because of their divergent adaptations, they are for the most part microallopatric in these areas (Krakauer 1970). Krakauer (1970) speculated that the existence of hybrid populations of these two ecologically distinct groups would be transient, as any potential advantage resulting from heterosis would be counterbalanced by maladaptation of hybrids to freshwater or saline environments.

The ecological distinctness of these snakes has resulted in controversy regarding their taxonomic status (Clay 1938, Cliburn 1960, Conant 1963), which may be clarified using molecular markers. Analyses of allozymes at 33 protein coding loci by Lawson et al. (1991) showed that except for areas of considerable habitat disturbance, gene flow between the saltmarsh and freshwater groups is very slight or absent and is on the same order as that seen between the freshwater group and *Nerodia sipedon*. Based on these analyses, they recommended elevation of the two groups to species level.

According to Lawson et al. (1991), the evolution of saltwater adaptation in these snakes took place on Floridian Pliocene islands. These islands were probably small and may have been devoid of standing fresh water with a topography and climate that resembled that of the Florida Keys today. They hypothesize expansion and colonization along the shores of the Gulf of Mexico followed the closure of the Suwannee Straits. The freshwater *Nerodia fasciata pictiventris* evolved through a southward expansion into peninsular Florida from an ancestral population that was originally distributed north of the Suwanee Straits (Lawson et al. 1991). If one accepts that the freshwater and saltmarsh groups evolved in allopatry, the transition zones described in Lawson et al. (1991) are the result of secondary contact. The ecological adaptations of saltmarsh snakes and the freshwater group have reached a high degree of specialization and the fusion of these two groups seems unlikely. Rather, with the passage of time, Lawson et al. (1991) expected that the selection gradient between the two habitats would eventually promote development of increasing specialization and further the divergence between them.

Genetic structure and within species processes

In contrast to the large divergence observed at allozyme loci between the freshwater and saltwater group, Lawson et al. (1991) found that the within-group divergence is minimal at the allozyme level. Two morphological characters, head shape and numbers of dorsal scale rows, unite the saltmarsh group *Nerodia fasciata clarkii, N. f. compressicauda,* and *N. f. taeniata* and distinguish them from the freshwater subspecies of *N. fasciata*. The molecular evidence supports the close association of these three saltmarsh forms, but the question of whether *N. f. taeniata* arose from ancestral *N. f. clarkii* or ancestral *N. f. compressicauda* could not be resolved by analyses of these data. The genetic distances separating the three taxa are no greater than those found between demes within each.

The Carolina salt marsh snake

The Carolina salt marsh snake (*Nerodia sipedon williamengelsi*), currently listed by the state of North Carolina as a taxon of special concern, is a melanistic water snake endemic to estuarine habitats in coastal North Carolina. It is closely associated with saltmarshes dominated by black needlerush (*Juncus romerianus*), and *Spartina* marsh grasses. Although no formal physiological studies have been conducted to determine if this subspecies has similar adaptations to saline environments as those mentioned above for the saltmarsh group, Conant and Lazell (1973) determined that Carolina salt marsh snake would not drink salt water, a finding similar to that of the *N. f. clarkii*.

In order to clarify the taxonomic status and genetic distinctness of the Carolina salt marsh snake, Gaul (1996) used a combination of molecular and morphological techniques to examine the relationships between this saltmarsh snake and the nominate subspecies *Nerodia sipedon sipedon*, as well as the dynamics of hybridization between the saltmarsh snake and a closely related species, the banded water snake (*Nerodia fasciata*). In a study of restriction endonuclease digests of mtDNA, Gaul (1996) found six unique haplotypes in coastal *N. sipedon*, but no clear distinction was detected between *N. s. williamengelsi* and *N. s. sipedon*. However, analysis of morphological characters revealed statistically significant differences between the two subpecies in numbers of ventral scales, subcaudal scales, and lateral bars. Two morphological characters, ventral scales and lateral bars, showed evidence of clinal variation and appear to correspond closely to estuarine salinity gradients. Evidence for hybridization between *Nerodia sipedon williamengelsi* and *N. fasciata* was observed in five specimens; mtDNA variation observed in these hybrids suggests that hybridization events between these two

species are bi-directional. In his review of the *N. sipedon-fasciata* complex, Conant (1963) speculated that interbreeding between the two forms represented introgressive hybridization, resulting from habitat alteration rather than evidence of conspecificity. However, further studies need to be done to establish the phylogenetic relationships of this subspecies. This is an example in which no evidence of genetic differentiation from a freshwater ancestor has occurred, despite significant morphological, ecological, and physiological adaptations.

CLAPPER RAIL AND KING RAIL

Systematics, distribution, and ecology

Clapper Rails are characteristic of tidal-salt and brackish-marsh habitat along the coasts of North America—and locally in freshwater marshes along the Colorado River and in the Imperial Valley, extending into mangrove swamps as far south as Peru and Brazil (Eddleman and Conway 1998). The closest inland relative to the Clapper Rail is the King Rail, which inhabits freshwater and brackish marshes, swamps, and rice fields (Ehrlich et al. 1988). Clapper Rails form a superspecies with the King Rail and another close relative, the Plain-flanked Rail (*Rallus wetmorei*), which occupies saltmarsh and mangrove habitats syntopically with Clapper Rails in Venezuela. Based on mitochondrial ATPase8 sequences from a museum specimen, *R. wetmorei* falls within a clade containing both King and Clapper rail sequences (B. Slikas, pers. comm.), and in fact is identical in sequence to King Rail and many Clapper Rails. No other *Rallus* species appear to be closely related to this group of rails.

Clapper Rails hybridize readily with King Rails in habitats of intermediate salinity on the East and Gulf coasts (Meanly and Weatherbee 1962) and the California subspecies of Clapper Rails were at one time considered subspecies of King Rails. Some authors, such as Ripley (1977) favor conspecific status, but the American Ornithologists' Union (1983) favored recognition as distinct species. Olson (1997) believed that the hybridization is limited to areas of intermediate salinity and does not result in enough introgression to justify merging of the taxa into one species. He showed that the width of the interorbital bridge, the region of the skull in which the salt glands occur, was greater in King than in Clapper rails. Furthermore, Olson provided evidence that this trait was stable and genetically based by comparing interorbital widths between Clapper Rails reared in captivity in freshwater with wild ones from salt-

marshes. He found the width was intermediate in one hybrid specimen.

Evolutionary history and biogeography

King and Clapper rails from the Gulf Coast showed very low levels of divergence in both allozymes (37 loci) and mtDNA (15 endonucleases; Avise and Zink 1988; King Rail, N = 10; Clapper Rail, N = 7). Allozymes showed no fixed differences and only one significant difference in allele frequency between the two species. The low allozyme divergence is expected given what has been found in birds generally (Barrowclough 1983), but the lack of divergence at mtDNA was surprising. Using 15 endonucleases Avise and Zink (1988) found an estimated sequence divergence of 0.6% in the mtDNA between King and Clapper rails. Interestingly, they also found evidence of intraspecific size polymorphism and more than one type per cell (heteroplasmy) in their mtDNA.

The low sequence divergence in King and Clapper rails has been confirmed by further research (R. C. Fleischer, unpubl. data). King and Clapper rails differed by 0.8% in cytochrome *b* sequences (while both differed from Virginia Rails [*Rallus limicola*] by 7–8%) and a tree rooted by the Virginia Rail showed the King Rail falling within the Clapper Rail clade. King and Clapper rails differed by an average of 0.3% for the central domain of the control region (275 base pairs), but King Rails (N = 4) had sequences identical to those of Gulf Coast (N = 4) and East Coast (N = 4) Clapper Rails. Thus the differences between the two species are minor and generally at the level of variation often found within populations of a single species. These results suggest that the morphological and ecological differences noted by Olson (1997) and others may be very recently evolved, or are maintained despite a large level of hybridization.

Conservation genetics

Twenty-one subspecies of Clapper Rails are described, with six occurring in the United States. The 21 subspecies have been divided into three groups based on plumage and geography: *crepitans* on the Atlantic and Gulf coasts of North America and the Caribbean; *obsoletus* in California and Northern Mexico; and *longirostris* in South America. Although most East Coast populations are abundant, West Coast populations are limited by substantial recent losses of habitat and three subspecies in the western US are listed as endangered (California Clapper Rail [*Rallus longirostris obsoletus*],

Light-footed Clapper Rail [*R. l. levipes*], and Yuma Clapper Rail [*R. l. yumanensis*]; Eddleman and Conway 1998).

In order to clarify the taxonomic status of the endangered subspecies of Clapper Rails, R. C. Fleischer (unpubl. data) compared mtDNA sequence variation between *Rallus longirostris levipes* (N = 7) and *R. l. yumanensis* (N = 4) of southern California and among samples from the subspecies *R. l. obsoletus* (N = 3) of northern California, *R. l. crepitans* of the eastern US (N = 4), and *R. l. saturatus* of the Gulf of Mexico (N = 4). No differences in central domain control region or cytochrome *b* mtDNA sequences were found among *R. l. yumanensis*, *R. l. obsoletus,* and *R. l. levipes*. No differences were found between *R. l. crepitans* and *R. l. saturatus*. Only a single base difference (in the control region sequence) separated the eastern and western samples indicating a divergence of <0.2% across the continent for the two gene regions. Thus mtDNA data provide little support for any of the subspecies occurring in North America.

Fleischer et al. (1995) and Nusser et al. (1996) also studied the genetics of two of these subspecies, *Rallus longirostris yumanensis* and *R. l. levipes* using minisatellite and randomly amplified polymorphic DNA (RAPD) markers to examine the extent of genetic variation within populations. They assessed variation among four disjunct marsh populations ranging from San Diego to Point Mugu along the southern California coast.

Fleischer et al. (1995) found extremely low minisatellite variation within the four isolated coastal marsh *Rallus longirostris levipes* populations (estimated heterozygosities of 25–42%), while the single sample of *R. l. yumanensis* yielded a more typical heterozygosity of 72%. Interestingly, the band-sharing among populations indicated a very low level of divergence (high gene flow) among the four coastal populations, but a substantially lower level of gene flow between the two subspecies (Fleischer et al. 1995). The RAPD analysis of the same birds (Nusser et al. 1996) revealed extremely low levels of variation in both subspecies (16 polymorphic bands out of 1,338 scored), and almost no divergence between them taking all loci into account (0.23% divergence). Patterns based on the 16 polymorphic bands revealed greater similarity among the four *R. l. levipes* populations than between these populations and *R. l. yumanensis*.

In summary, members of the *Rallus longirostris-elegans* complex are found across North America and exhibit limited divergence between species and among subspecies of Clapper Rails. A comprehensive study that looks further at relationships within and between both King and Clapper rails is needed to investigate the hypothesis of the invasion of Clapper Rails into the tidal-marsh habitat and morphological and genetic differentiation from populations of King Rails. An investigation of the degree of hybridization and back-crossing between the two species is also important. Additional genetic markers, such as microsatellites or AFLPs (amplified fragment length polymorphism) need to be developed to allow us to determine the patterns of colonization of North America. Furthermore, genetic studies indicate that isolated coastal populations of *R. l. levipes* exhibit low levels of heterozygosity and may therefore be in danger of reduced viability and inbreeding depression.

SALTMARSH SHARP-TAILED SPARROW AND SEASIDE SPARROW

Systematics, distribution, and ecology

The genus *Ammodramus* contains the only two species of bird that are essentially endemic to tidal marshes (Greenberg and Maldonado, *this volume*): the Saltmarsh Sharp-tailed Sparrow and the Seaside Sparrow. We use the term essentially, because the latter species has some subspecies, including the extinct Dusky Seaside Sparrow (*Ammodramus maritimus nigrescens*) in east Florida and the endangered Cape Sable Seaside Sparrow (*A. m. mirabilis*) in south Florida that occurred or occur locally in freshwater marsh and flooded prairie as well as brackish and saltmarsh. Nelson's Sharp-tailed Sparrow (*A. nelsoni*), which until recently (American Ornithologists' Union 1983) was considered conspecific with the Saltmarsh Sharp-tailed Sparrow, also has two subspecies that are associated with coastal marshes.

Taxa within the seaside-sharp-tail group of sparrows have received various levels of taxonomic recognition. Different subspecies or groups of subspecies have been elevated to or demoted from species status. As many as five and as few as two species have been recognized. In this account, we capitalize the names of the three currently recognized species, and sharp-tailed sparrows refers to Nelson's and Saltmarsh Sharp-tailed sparrows together. Figure 1 shows species names and relationships.

The Seaside Sparrow breeds in the Gulf and Atlantic coast saltmarshes from New Hampshire south to Florida and west to Texas. Nine subspecies of Seaside Sparrow are currently recognized, of which two were formerly accorded species status (American Ornithologists' Union 1957, 1973), but it is likely that the number
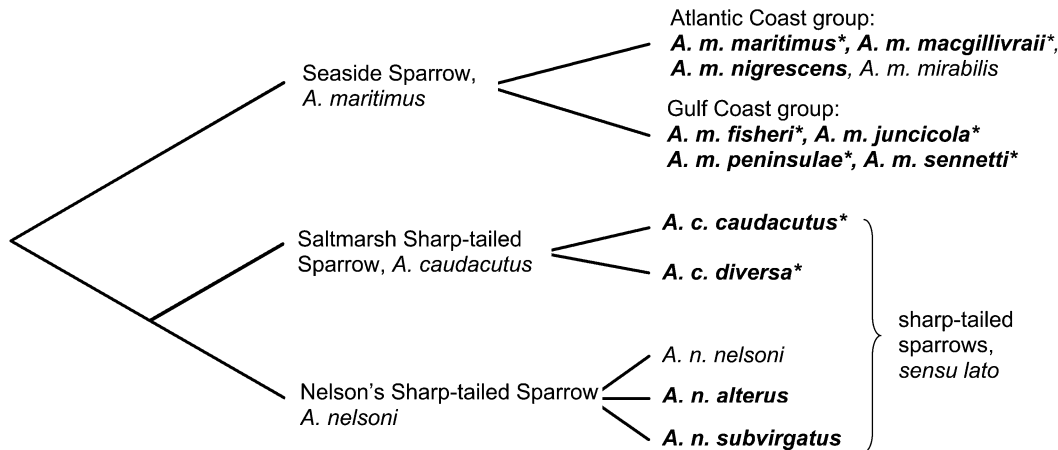
FIGURE 1. Relationships among species and subspecies of marsh-nesting *Ammodramus* sparrows. Taxa in bold inhabit tidal marshes; tidal-marsh obligates are indicated with asterisks. *A. nelsoni nelsoni* inhabits mid-continent freshwater marshes and *A. melodia mirabilis* inhabits freshwater prairies in Florida. Major mtDNA lineages in sharp-tailed sparrows (1.2% different; Rising and Avise 1993) are currently recognized as full species, while major mtDNA lineages in Seaside Sparrows (1.0%; Avise and Nelson 1989) are not, and are labeled groups above. Although this diagram is consistent with existing molecular data (J. Klicka, unpubl. data), it is provided primarily to aid the reader in following the more detailed discussion of named taxa.

of recognized subspecies should be reduced (Post and Greenlaw 1994). Populations from New Jersey north (*Ammodramus maritimus maritimus*) are largely migratory and winter in the southeastern US. South of the Chesapeake Bay, Seaside Sparrow populations are year-round residents and include the subspecies *A. m. macgillivraii* from the mid-Atlantic states to Georgia, *A. m. nigrescens* and *A. m. mirabilis* in east and south Florida respectively, and *A. m. peninsulae, A. m. juncicola, A. m. fisheri* and *A. m. sennetti* along the Gulf Coast from Florida to Texas. With the exception of the subspecies on the Florida Peninsula, Seaside Sparrows are saltmarsh obligates, breeding, wintering, and even migrating via saltmarsh habitat.

The Saltmarsh Sharp-tailed Sparrow breeds in saltmarshes from southern Maine to Virginia. Two weakly differentiated subspecies are recognized: *Ammodramus caudacutus caudacutus* from Maine to New Jersey and *A. c. diversus* from New Jersey to the Delmarva Peninsula. Most individuals migrate to the southern US to winter along the Atlantic Coast and, rarely, the Gulf Coast (Post 1998). Saltmarsh Sharp-tailed Sparrows are also saltmarsh obligates, and their range is as linear and patchy as that of the Seaside Sparrow.

Nelson's Sharp-tailed Sparrow encompasses three subspecies, one of which, *Ammodramus nelsoni nelsoni*, breeds in interior freshwater marshes. A second, *A. n. alterus*, breeds in coastal marshes along James Bay and Hudson

Bay. The third, *A. n. subvirgatus,* breeds from the Gulf of St. Lawrence to southwestern Maine. *A. n. subvirgatus* breeds mostly in saltmarshes, but perhaps partly due to differences in habitat availability in the northern part of its range, can be found in brackish and fresh estuarine marshes as well. All three subspecies are migratory, wintering in the coastal marshes of the southeastern US, with the interior subspecies wintering largely along the Gulf Coast (Post 1998; J. S. Greenlaw, pers. comm.).

Evolutionary history, and biogeography

The systematics, biogeography, and degree of population isolation of the *Ammodramus* sparrows has been addressed repeatedly in the past century using morphology, behavior, and molecular genetic tools such as allozymes, mtDNA RFLPs, microsatellite DNA polymorphism and mtDNA sequencing. This offers the possibility of understanding timing and evolutionary dynamics involved in what may have been multiple transitions by upland forms to tidal marsh endemism.

Zink and Avise (1990) examined the phylogenetic relationship of eight *Ammodramus* species based on trees derived from mtDNA RFLPs (16 restriction enzymes) and allozymes (30 enzyme loci, 24 variable), and using the Savannah Sparrow as an outgroup. LeConte's (*A. lecontei*), Seaside and sharp-tailed sparrows consistently formed a wetlands clade in this

analysis (Rising 1996). Although the Savannah Sparrow may not be an appropriate outgroup to *Ammodramus* as a whole (Avise et al. 1980, Zink and Avise 1990, Carson and Spicer 2003) (*Ammodramus* as currently constituted is probably not monophyletic), Zink and Avise's (1990) work provides the best framework so far for understanding the relationships within the genus. MtDNA data and allozyme data differed on the exact relationships within the wet grassland clade so it remains unclear if sharp-tails share a more recent common ancestor with LeConte's or Seaside sparrows.

Prior to the Zink and Avise phylogenies, two scenarios were proposed for the evolution of the saltmarsh *Ammodramus*. Beecher (1955) invoked late Pleistocene and Holocene isolation by post-glacial marine embayments that formed over depressed river valleys as the mechanism leading to diversification of the Seaside from sharp-tailed sparrows, which in turn were derived from Savannah Sparrows. Funderburg and Quay (1983) suggested that Seaside Sparrows evolved from Savannah Sparrows in the late Pliocene and early Pleistocene.

In a scenario more consistent with the molecular phylogenies Greenlaw (1993) proposed two cycles of vicariance and differentiation in sharp-tailed sparrows in the Pleistocene. Depending on whether sharp-tails were derived from Seaside or LeConte's sparrows, Greenlaw presents two alternative scenarios. In the first, Seaside Sparrows invade tidal habitats on the East Coast and later give rise to the southern coastal form of sharp-tail (now *Ammodramus caudacutus*), which then colonizes inland, freshwater habitats, evolving into *A. nelsoni*. The inland form then reinvades coastal habitat at a later time, becoming the present-day subspecies *A. n. subvirgatus*. This scenario involves two invasions of tidal habitat—an early invasion by Seaside Sparrows, and a very recent reinvasion of saltmarshes by *A. n. subvirgatus*.

Greenlaw's alternative scenario posits LeConte's Sparrow as the sister taxon to sharp-tails. In this scenario, LeConte's Sparrow gives rise to an inland, freshwater form of sharp-tail (now *Ammodramus nelsoni*). This form invades coastal habitats in mid-Pleistocene and gives rise to *A. caudacutus*. Later, the inland form invades tidal habitats a second time, giving rise to *A. n. subvirgatus*. This second scenario involves three separate invasions of tidal habitat by *Ammodramus* sparrows: (1) the inland form of the sharp-tail gives rise to *A. caudacutus*, (2) the inland form later gives rise to *A. n. subvirgatus*, and (3) Seaside Sparrows invade tidal marshes independently of sharp-tails. In both cases, the current zone of sympatry between

A. n. subvirgatus and *A. c. caudacutus* in southwestern Maine is hypothesized to represent secondary contact.

The uncertain phylogeny of the *Ammodramus* genus as a whole complicates speculation about the invasion of tidal marsh habitat by Seaside and/or sharp-tailed sparrows. The ancestral condition of emberizines is terrestrial—at least one invasion of tidal marshes must have occurred. If contact between *A. n. subvirgatus* and *A. c. caudacutus* is secondary, and if *A. n. subvirgatus* is descended from inland forms, as put forth by Greenlaw (1993) at least two invasions of tidal marshes have occurred. If sharp-tailed sparrows share a more recent common ancestor with LeConte's Sparrows than with Seaside Sparrows, then three separate invasions may have occurred. At this point the genetic evidence appears to rebut the scenarios that hypothesize a close link between Seaside and Savannah sparrows. At the very least, Seaside Sparrows are more closely related to both LeConte's and sharp-tailed sparrows than they are to Savannah Sparrows. However, the exact order of branching within the LeConte's-Seaside-sharp-tailed clade is not yet clear, nor is the closest relative outside that group. To some extent we are still awaiting a more comprehensive phylogeny of the Emberizidae that includes the marsh-nesting *Ammodramus* species in the context of close and distant relatives before we can distinguish between some of these scenarios, and put speculation about the original shift(s) by the ancestor(s) of these species to tidal marsh endemism on more solid ground.

No source disputes that within the marsh-nesting sparrows the Nelson's Sharp-tailed Sparrow and the Saltmarsh Sharp-tailed Sparrow are each other's closest relatives. In fact, until recently the two forms were considered conspecific (American Ornithologists' Union 1983). Greenlaw (1993) documented both morphological (plumage coloration, bill size) and behavioral (song and display) differences between a northern group of sharp-tailed sparrows (the subspecies *nelsoni*, *alterus*, and *subvirgatus*, at that time still part of *Ammodramus caudacutus*) and a southern group (subspecies *caudacutus* and *diversus*). Rising and Avise (1993) used mtDNA RFLPs and skeletal morphology to study differences among the subspecies and populations of sharp-tailed sparrows (N = 220 individuals, 12 sites throughout the range, 20 restriction enzymes) and discovered a deep division (average of 1.2% sequence divergence) between northern and southern forms, and much less divergence (0.2%) within each region. Assuming a rate of mtDNA RFLP sequence divergence of 2%/1,000,000 yr (Shields and

Wilson 1987), the gap between northern and southern forms corresponds to a separation of approximately 600,000 yr duration. The mtDNA groups correspond to Greenlaw's behavioral division between northern and southern forms, and skeletal characters also distinguished northern from southern sharp-tails (Rising and Avise 1993). Also, observations from marshes in southern Maine, where the northern mtDNA forms (represented by *A. n. subvirgatus*) and the southern forms (*A. c. caudacutus*) come in contact, suggested that assortative mating is the rule where the forms are sympatric (Greenlaw 1993).

Following these studies, the American Ornithologists' Union (1995) recognized each form as a full species, the northern form as Nelson's Sharp-tailed Sparrow and the southern as Saltmarsh Sharp-tailed Sparrow. The presumed date of the split based on mtDNA differences between these two species (600,000 yr before present, mid-Pleistocene) is inconsistent with the hypothesis put forward by Beecher (1955), who had proposed the early Holocene (about 8,000 yr before the present) as the date of sharp-tailed sparrow diversification into present forms. Likewise, the division of Seaside Sparrows into two similarly ancient groups argues against the timing suggested by Beecher (1955) and Funderburg and Quay (1983) for late Pleistocene-Holocene diversification of Seaside Sparrows.

Genetic structure and within-species processes

In the same way that sharp-tailed sparrows proved to have a deep genetic divide between two groups, Seaside Sparrows also show a major genetic split. However, unlike in sharp-tails, the genetic divide in Seaside Sparrows does not appear to be consonant with any morphological or behavioral divide. In a broad-ranging survey, Avise and Nelson (1989) assayed 40 individuals from 10 locations throughout the Seaside Seaside Sparrow's range, digesting mtDNA with 18 informative restriction enzymes. Nelson et al. (2000) assayed mtDNA of four individuals of the threatened Cape Sable Seaside Sparrow (*Ammodramus m. mirabilis*) with the same enzymes. These studies found a group of Atlantic Coast birds that was quite divergent in mtDNA genome from a second group along the Gulf Coast. Mitochondrial sequence divergence was estimated at 1% between the two groups, corresponding to separate evolutionary trajectories for these two groups over the last 500,000 yr (Avise and Nelson 1989). Sequence divergence among individuals on each coast averaged about 0.2%. Both of the isolated

and distinctively marked Florida forms, the endangered *A. m. mirabilis* and the extinct *A. m. nigrescens*, belonged to the Atlantic Coast group (Avise and Nelson 1989, Nelson et al. 2000), and in fact, nothing from mtDNA typing suggested that either subspecies was particularly divergent from other Atlantic Coast forms.

Because of the concordance between phenotypic and molecular characters, the American Ornithologists' Union (1995) now recognizes two species of sharp-tailed sparrows. With only the mtDNA divergence data showing an ancient divergence, the Gulf and Atlantic coast populations of Seaside Sparrows have not been accorded species status, and in fact the divergence between Atlantic and Gulf coast Seaside Sparrows receives no special taxonomic recognition at all. MtDNA is well suited to tracing gene genealogies, but multiple nuclear loci are particularly useful in monitoring gene diversity and gene flow between populations. The patchy and isolated nature of tidal-marsh habitats and the small population sizes within patches may make saltmarsh sparrows particularly prone to loss of genetic diversity due to bottlenecks. Seutin and Simon (1988) observed one example of this effect when perhaps as few as three individuals of *subvirgatus* type Nelson's Sharp-tailed Sparrows established a breeding colony on fresh water in the St. Lawrence River, Quebec, in 1980, 200 km from the nearest known breeding site. This colony increased to perhaps 50 individuals over the next 6 yr. Three males collected in 1986 displayed complete uniformity at 46 enzyme loci, and also showed no variation in protein banding patterns from isoelectric focusing (Seutin and Simon 1988).

C. E. Hill (unpubl. data) examined population differentiation in three populations of Seaside Sparrows (N = 15–61 individuals/population), using four microsatellite loci. One of those populations was from St. Vincent National Wildlife Refuge in Apalachicola, Florida (representing the Gulf Coast mtDNA group), and two were from South Carolina, from marshes near Georgetown and near Charleston (about 90 km from each other, and roughly 600 km from the Florida population). As would be predicted by the mtDNA studies or by an isolation-by-distance model, the two South Carolina populations were more similar to each other than either was to the Florida population. However, the two South Carolina populations also showed significant differences from each other in allelic frequencies at microsatellite loci, even though separated by less than 100 km, suggesting low levels of gene flow between Seaside Sparrow populations even over short distances.

Conservation genetics

Understanding the genetic structure of *Ammodramus* sparrow populations can guide the captive breeding of highly endangered forms, reveal which populations are affected by hybridization, and also help in setting conservation priorities for less threatened forms. Knowledge of the genetic divide between Gulf and Atlantic coast Seaside Sparrows would have better informed the selection of a breeding female to mate with the only surviving Dusky Seaside Sparrow. We now know that Dusky Seaside Sparrows had Atlantic, not Gulf, mtDNA and that, had the male Dusky Seaside Sparrows survived long enough to complete the breeding program, all the offspring from the captive breeding program would have had Gulf Coast mtDNA (Avise and Nelson 1989), although having the wrong mtDNA might not have hurt the viability of those offspring (Zink and Kale 1995).

In another genetic study with conservation implications, Shriver (2002) used five polymorphic microsatellite loci to estimate gene flow across the zone of sympatry of Nelson's (*Ammodramus n. subvirgatus*) and Saltmarsh (*A. c. caudacutus*) Sharp-tailed Sparrows in southern Maine. He sampled sparrows from five breeding locales, ranging from Lubec, Maine (allopatric *A. n. subvirgatus*), to three marshes in southwestern Maine, where both species occur, to Prudence Island, Rhode Island (allopatric *A. c. caudacutus*). In the area of sympatry, he classified 19 of 89 birds by plumage as hybrids. Of the apparently pure parental types in the zone of sympatry, 29 birds appeared to be Nelson's Sharp-tailed Sparrows, and 41 appeared to be Saltmarsh Sharp-tailed Sparrows. Genetic analysis confirmed that the hybrids were intermediate in allelic frequencies between the allopatric parental forms. In addition, the putative parental types within the zone of sympatry carried, on average, 25% of their microsatellite genotype from the other species. Few genetic barriers apparently exist between the two newly recognized species of sharp-tailed sparrows. Introgression may be slowed by the narrow nature of the hybrid contact, but *A. n. subvirgatus* genes have still been found in *A. c. caudacutus* populations as far south as Parker River, Massachusetts, which Shriver argues should perhaps increase the conservation priority for Saltmarsh Sharp-tailed Sparrows in the area unaffected by introgression (Shriver 2002).

The genetic exchange across the hybrid zone between the two species of sharp-tailed sparrows also brings back unanswered questions about the biogeography of sharp-tailed and Seaside Sparrows in the Pleistocene glaciations. If the two mtDNA defined clades of sharp-tails interbreed so readily today, then how have the two clades maintained their distinctiveness through 600,000 yr and several Pleistocene glacial advances? Were the two groups confined to distinct refugia in each glacial advance and have only come into contact in the Holocene? Perhaps the ancestors of Nelson's Sharp-tailed Sparrows were in freshwater refugia and the ancestors of Seaside Sharp-tailed Sparrows on the coast (Greenlaw and Rising 1994). The same question could be asked about the Gulf Coast versus Atlantic Coast seaside sparrow mtDNA groups: how did they remain separate through 500,000 yr of Pleistocene glaciation and of sea-level rise and fall, when the distribution of salt-marsh habitat was radically different from the present distribution?

In summary, the Seaside Sparrow, the Saltmarsh Sharp-tailed Sparrow, and the *subvirgatus* subspecies of the Nelson's Sharp-tailed Sparrow have all invaded the tidal-marsh habitat and are fully or largely endemic to it. Since their invasion of saltmarshes, the forces that have determined their evolutionary trajectories may have included the linear and patchy nature of their habitat and a corresponding tendency to isolation in small habitat islands, as well as apparent strong selection, especially on plumage color. The role of glacial advances and the ways in which the sparrows were assorted into refugia during times of glacial advance are not entirely clear. There are divisions apparent from mtDNA analyses dating back 500,000–600,000 yr in both Seaside and sharp-tailed sparrows. Within each mtDNA group, subspecies are distinctive at least in plumage (Seasides), and sometimes in plumage, ecology, and morphology (sharp-tails). The two species of sharp-tails retain their ability to interbreed, and do interbreed in New England; the two mtDNA groups of Seaside Sparrows are currently allopatric. One subspecies of Seaside Sparrow is extinct, another is endangered, and the restricted and fragmented range of all these birds means local populations or entire subspecies may be quite vulnerable to extirpation, as happened with the Dusky Seaside Sparrow.

SAVANNAH SPARROW

Systematics, distribution, and ecology

Savannah Sparrows range across North America from Alaska to central Mexico and are characteristic of open habitats including grasslands, meadows, and agricultural fields. Seventeen subspecies are recognized. Although

most populations of Savannah Sparrows are migratory, five or six of the subspecies are resident or partially migratory in saltmarshes in California or Mexico and two are resident in coastal Sonora and Sinaloa (Wheelwright and Rising 1993, Rising 2001). These tidal-marsh subspecies can be divided into two groups, the large-billed group (*Passerculus sandwichensis rostratus*) and the Belding's group (*P. s. beldingi*). The large-billed Savannah Sparrows are distinctive enough morphologically to have once been considered a species (American Ornithologists' Union 1931). They are characterized by a large decurved culmen and are pale with diffuse streaking (Taber 1968b, Pyle 1997). Tidal marsh subspecies belonging to the large-billed group consist of *P. s. rostratus* (breeds on the Gulf Coast of northeast Baja California and northwest Sonora) and *P. s. atratus* (resident on the coast of central Sonora south to central Sinaloa). The Belding's group is characterized by long, straight, slender bills and is darkly streaked (Taber 1968a, Pyle 1997). The Belding's group consists of *P. s. beldingi* (resident on the Pacific coast from Morro Bay, California, south to El Rosario, Baja California), *P. s. annulus* (resident around the shores of Bahia Sebastian Vizcaino, Baja California) and *P. s. guttatus* (resident around Laguna San Ignacio) and *P. s. magdalenae* (resident around Bahia Magdalena; Wheelwright and Rising 1993). Breeding Savannah Sparrows increase clinally in size along the Pacific Coast from south to north (Rising 2001).

Genetic differentiation of tidal-marsh endemic

In a preliminary molecular study, Zink et al. (1991) compared three specimens of *Passerculus sandwichensis rostratus* using 20 restriction endonucleases and found a large amount of genetic differentiation between *P. s. rostratus* and non-tidal marsh subspecies in California (N = 3) and Louisiana (N = 5). They estimated 1.7% sequence divergence between non-marsh and *P. s. rostratus* (Zink et al. 1991). In a more comprehensive study Zink et al. (in press) sequenced two mitochondrial genes, ND2 and ND3 from five sites in Baja California, and coastal Sonora, and compared them with eight continental populations (total N = 112). They found the saltmarsh populations to be genetically distinct from continental populations (average nucleotide sequence divergence was 2%, F_{st} = 0.063, P < 0.001). They recommended based on genetic, morphological, and behavioral differences that the saltmarsh and typical Savannah Sparrows be considered separate species (Zink et al., in press).

Zink et al. (in press) further suggested that at one time three isolated populations of Savannah Sparrows existed, belonging to three clades. Since then, two of the clades which are found in continental Savannah Sparrows have become admixed, however the third clade belonging to the saltmarsh Savannah Sparrows have remained isolated either by habitat barriers or geographical distance. Using a calibration of 2% sequence divergence per million years (Shields and Wilson 1987, Tarr and Fleischer 1993), *P. s. rostratus* has been isolated for at least 750,000 yr from typical populations of Savannah Sparrows (Zink et al. 1991). The lack of geographic structure within saltmarsh Savannah Sparrows suggests that there is either widespread gene flow throughout the range of saltmarsh Savannah Sparrows, or that the colonization of different saltmarsh habitats are too recent for effective sorting of lineages (Zink et al. in press).

Song Sparrow and Swamp Sparrow

Sparrows of the genus *Melospiza* are widespread in shrubby habitats and wetlands throughout North America. Of the three species in the genus, the Song Sparrow (*Melospiza melodia*) and the Swamp Sparrow (*M. georgiana*) have colonized tidal-marsh habitats and have recognized tidal marsh subspecies along the shores of San Franciso Bay and the mid-Atlantic estuaries, respectively. The Swamp Sparrow probably split (along with the boreal-breeding Lincoln's Sparrow [*Melospiza lincolnii*]) from a common ancestor shared with Song Sparrows in the early to mid-Pleistocene (Zink and Blackwell 1996).

Systematics, distribution, and ecology

Song Sparrows are widespread inhabitants of moist habitats across North America. Extremely variable across their range, various authorities have recognized from 24–30 diagnosable subspecies (American Ornithologists' Union 1957, Arcese et al. 2002). Three of those subspecies are resident in tidal-saltmarsh habitat in the San Francisco Bay region, each occupying one of three sub-bays of the greater San Francisco Bay. The Samuel's Song Sparrow (*Melospiza melodia samuelis*) is resident in San Pablo Bay, the Suisun Song Sparrow (*M. m. maxillaris*) in Suisun Bay, and the Alameda Song Sparrow (*M. m. pusillula*) in south San Francisco Bay (Fig. 2). These Song Sparrow subspecies differ markedly from each other and related non-tidal marsh subspecies in plumage and size. The Samuel's Song Sparrow is small in size and blackish olive in dorsal coloration (Marshall 1948b). The Alameda Song Sparrow is slightly smaller than the Samuel's
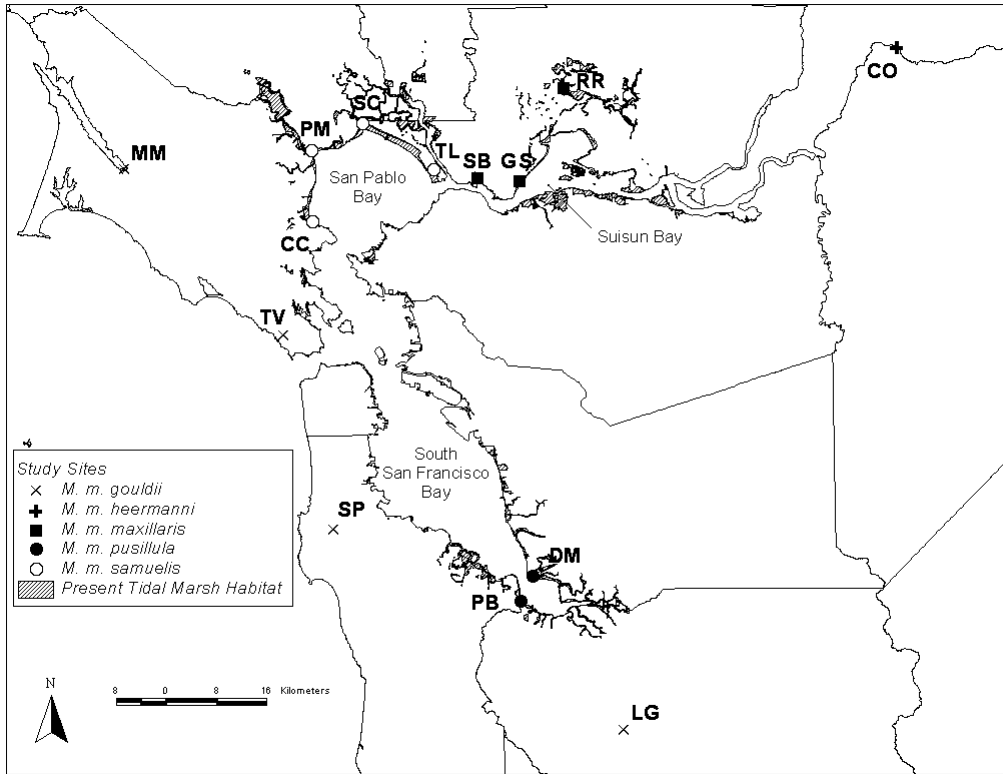
FIGURE 2. Map of San Francisco Bay region showing sampling sites from Chan and Arcese (2003) and the tidal-marsh habitat of three tidal-salt-marsh subspecies of Song Sparrow (*M. melodia samuelis, M. m. maxillaris,* and *M. m. pusillula*). The range of *M. m. samuelis* is San Pablo Bay (sites—China Camp State Park, Marin Co. [CC], Petaluma River Mouth, Sonoma Co. [PM], Sonoma Creek, Sonoma Co. [SC], and Triangle Levy, Sonoma Co. [TL]), *M. m. maxillaris* is Suisun Bay (sites—Benicia State Recreation Area, Solano Co. [SB], Goodyear Slough, Solano Co. [GS], and Rush Ranch Open Space, Solano Co. [RR]), and *M. m. pusillula* is San Francisco Bay (sites—Palo Alto Baylands, Santa Clara Co. [PB], Dumbarton Marsh, Alameda Co. [DM]). *M. m. gouldii* occurs in upland habitats surrounding the bay (sites—Mark's Marsh, Marin Co. [MM], Tennessee Valley, Marin Co. [TV], San Pedro Valley County Park, San Mateo Co. [SP], and Los Gatos Creek County Park [LG]); *M. m. heermani* occurs to the east of Suisun Bay (site—Cosumnes River Preserve, Sacramento Co. [CO]) (San Francisco Estuary Institute. 2000).

Song Sparrow, has a yellowish-grey dorsal color and is the only Song Sparrow subspecies with a yellowish wash to the belly (Ridgeway 1899, Marshall 1948b). Suisun Song Sparrow is the largest of the tidal-marsh subspecies with a laterally flared bill at the nostrils (Marshall 1948b). The Suisun Bay birds have the largest relative bill size of any North American Song Sparrow. The closest upland species are the Marin Song Sparrow (*M. m. gouldii*) found along the California coast (Grinnell and Miller 1944) and the Heerman's Song Sparrow (*M. m. heermani*) resident in central and southwest California (previously *M. m. mailliardi*; Arcese et al. 2002) whose range meets the Suisun Song Sparrow at the eastern edge of Suisun Bay.

Swamp Sparrows are typical inhabitants of freshwater and brackish marshes across the eastern US and Canada (Mowbray 1997). In contrast to the extremely polytypic Song Sparrow, the Swamp Sparrow consists of only three subspecies, one of which, the Coastal Plain Swamp Sparrow (*Melospiza georgiana nigrescens*), is endemic to tidally influenced brackish marshes and freshwater marshes along the mid-Atlantic Coast (Wetherbee 1968). The Coastal Plain Swamp Sparrow is distinguished by a larger bill, grayer plumage, and increased black coloration on the head and nape (Greenberg and Droege 1990).

Evolutionary history and biogeography

Song Sparrows show no geographic structure in mitochondrial haplotypes corresponding to the geographic variation in plumage

and other aspects of morphology captured in the currently recognized subspecies (Zink and Dittmann 1993, Fry and Zink 1998). The lack of geographic structure in mitochondrial haplotypes may result from the expansion of the Song Sparrow from multiple refugia after the glaciers receded (Zink and Dittmann 1993, Fry and Zink 1998). To further explore the probable recency of the evolution of geographic variation in Song Sparrows in general, Chan and Arcese (2002) examined genetic structure within and among tidal-marsh populations. This analysis used nine microsatellite loci to examine differentiation among six tidal marsh populations (two per tidal marsh subspecies) and three nearby upland populations (total N = 215, average of 22 birds per population; Fig. 2). Overall genetic differentiation was extremely low (F_{st} = 0.0217, P < 0.001) indicating a large amount of gene flow (7.78 immigrants per generation; Chan and Arcese 2002). Despite the low amount of differentiation, they found the Alameda Song Sparrow was genetically distinct from both upland subspecies and other tidal-marsh subspecies. However, they did not find statistically significant differences in allele frequencies between the Samuel's Song Sparrow, Suisun Song Sparrow, and the Heerman's Song Sparrow (Chan and Arcese 2002). The lack of differentiation among those subspecies, coupled with the pattern of variation at microsatellite loci found by Chan and Arcese (2002) suggest that San Pablo Bay and Suisun Bay tidal-marsh sparrows were the result of a recent invasion from the Central Valley of California.

This hypothesis is concordant with the geological history of the tidal marshes in the San Francisco Bay region, which provide some insight into the evolutionary history of the tidal-marsh Song Sparrow subspecies. Evidence from core samples indicates at least four episodes of emergence and submergence from river valley to estuary on the continental shelf that is now San Francisco Bay (Atwater 1979). The most recent submergence occurred approximately 10,000 yr ago, when what is now San Francisco Bay was a riparian valley through which the Sacramento and San Joaquin rivers flowed (Atwater 1979). The current water level was reached about 5,000 yr ago (Atwater 1979), but the tidal marshes surrounding San Francisco Bay are probably 4,000–6,000 yr old with south San Francisco Bay marshes being a bit younger, approximately 2,000 yr old (Atwater et al. 1979).

The origin of the Alameda Song Sparrow is not clear because the sister group was not identified in their study; however, the pattern of relatedness between populations suggests a different colonization into the tidal marsh habitat for the Alameda Song Sparrow. Therefore, it appears that two separate invasions to the tidal-marsh habitat in San Francisco Bay occurred, not subspecific differentiation within a single colonization.

Despite the morphological divergence apparent in all three tidal-marsh Song Sparrow subspecies (Marshall 1948b; Chan and Arcese 2002, 2003), only one subspecies was differentiable at a neutral, rapidly evolving marker. Chan and Arcese (2002) noted four possible explanations for this discrepancy: (1) recent divergence of tidal marsh subspecies and lack of differentiation due to inadequate lineage sorting—possibly due to large effective population size, (2) high current gene flow and strong selection on morphological or plumage characters resulting in a decoupling of neutral loci and quantitative loci, (3) high current gene flow resulting in introgression between previously differentiated subspecies, or (4) high gene flow with a large environmental component to morphological and plumage development. Further research involving common garden experiments would aid greatly in differentiating between these scenarios.

As with Song Sparrow subspecies, molecular studies on the Coastal Plain Swamp Sparrow have failed to find marked differentiation at nuclear (Balaban 1988) or mitochondrial loci (Greenberg et al. 1998). Greenberg et al. (1998) sequenced a total of 641 base pairs of mtDNA, including the hypervariable mtDNA control region, COII/t-lys/ATPase8, and ND2. They found extremely low levels of genetic variation (mean sequence divergence = 0.21%) and population differentiation (F_{st} = 0.057, P = 0.208) from 29 Swamp Sparrows, including individuals from two populations of the Coastal Plain Swamp Sparrow (near Delaware Bay, N = 7; near Chesapeake Bay, N = 4), two populations of the two inland subspecies (Garrett County, Maryland N = 5; Clay County, Minnesota N = 2), and several migrant populations (total N = 11). More recent analyses of five microsatellite loci (R. C. Fleischer et al., unpubl. data) provide additional support for a very low level of genetic divergence between the Coastal Plain Swamp Sparrows and the inland subspecies.

The low level of variation indicates a recent coalescence of mtDNA haplotypes in Swamp Sparrows, which is estimated based on ATPase8 at 40,000 yr (Greenberg et al. 1998). Given that most of the current range of Swamp Sparrows was covered by glaciers in the past 10,000–15,000 yr, the Coastal Plain Swamp Sparrow appears to have differentiated morphologically in a very short amount of time (Greenberg et

al. 1998). The question of whether the morphological differentiation is mainly genetic or environmental remains problematic, but nestling Coastal Plain Swamp Sparrows that were hand reared in an aviary with only fresh water developed stereotypical plumage patterns and morphology (R. Greenberg, pers. comm.). Recent analyses of the melanocortin-1 receptor gene (L. Gibbs et al., unpubl. data), known to result in darker plumage patterns in a wide range of avian taxa (Theron et al. 2001, Mundy et al. 2004), have not revealed a relationship for this species.

In summary, morphological divergence among endemic tidal-marsh subspecies of *Melospiza* sparrows and their freshwater relatives appear to be recently evolved or strongly environmentally influenced. Little support was found for genetic differentiation of tidal marsh subspecies in this genus.

ORNATE SHREW AND WANDERING SHREW

Systematics, distribution, and ecology

Populations of both the ornate shrew (*Sorex ornatus*) and the wandering shrew (*Sorex vagrans*) are found in coastal marshes of the West Coast of North America. The ornate shrew is a rare species restricted to coastal marshes and riparian communities of California, from 39° N latitude southward discontinuously to the tip of Baja California, Mexico. Subspecies of the ornate shrew often were described in the past by body size and pelage coloration which may be the result of environmental induction rather than genetically based differences, and sometimes based on only one or two specimens (Owen and Hoffmann 1983). However, the validity of the nine named subspecies of ornate shrews has recently been confirmed using univariate and multivariate statistical analyses of cranial measurements (Maldonado et al. 2004). Wandering shrews occur in northwestern North America down the Coast Range and Sierra Nevada of northern California. At least one subspecies of wandering shrew is known from the marshes of San Francisco Bay. Because of their short life span (Rudd 1953, Newman 1976), semi-fossorial habit, habitat specialization, high metabolism (McNab 1991), and small size, dispersal between patches of mesic habitat is limited and the high degree of local geographic morphological and genetic variation in shrews is expected. Furthermore, faced with a high abundance of invertebrate food, shrew populations can achieve high local densities in tidal marshes. Therefore, the evolution of multiple saltmarsh subspecies in this genus is also not surprising.

Although the existence of nine morphologically distinct subspecies of ornate shrew is well founded, a molecular genetic analysis of this species using mtDNA and allozymes, separates the species into southern, central, and northern clades (Fig. 3). Hence the patterns of morphological and genetic variation in this species are not concordant. Furthermore, genetic analysis puts into question the species status of certain morphologically ascribed subspecies, in the San Francisco Bay marshes. Therefore, we will develop our analysis of the evolutionary relationship of these species based on a recent molecular analysis (Maldonado et al. 2001; Fig. 3).

Patterns of genetic differentiation in saltmarsh habitats

In the genetic analysis of the cytochrome *b* region of the mtDNA of ornate shrews, Maldonado et al (2001) found 24 different haplotypes in 20 populations. Except for three population groupings, all populations had unique haplotypes. The occurrence of unique haplotypes in most localities suggests that genetic subdivision is a common characteristic of ornate shrews throughout most of their range. Fourteen of the 20 populations of ornate shrews were fixed for a single unique haplotype (392 base pairs). The remaining six populations had two to four different haplotypes. Interestingly, two of the subspecies endemic to tidal marshes (Grizzly Island [*Sorex ornatus sinuosus*—population 2 in Fig. 2] with Rush Ranch [*S. o. californicus*—population 5], and Los Banos [*S. o. californicus*—population 8]) with Salinas [*S. o. salarius*—population 10]; Fig. 3) were not significantly differentiated from the more widely distributed subspecies and have all haplotypes in common, implying that they are part of the same interbreeding population. In addition, even though pairwise computations of F_{st} using an analysis of molecular variance (AMOVA) indicate that most ornate shrew populations are significantly differentiated relative to a random collection of genotypes, once again these two populations, restricted to tidal marshes, are included among those that are not genetically differentiated (Grizzly Island versus Rush Ranch, Salinas versus Los Banos, and Salinas versus Sierra Nevada). These populations were also not significantly differentiated in the allozyme analysis. Several populations from different groupings were geographically distant but showed small genetic distances. The clearest example occurs in the central populations where the Salinas population is approximately 300 km away from the Los Banos populations, but the average Nei's allozyme genetic distance
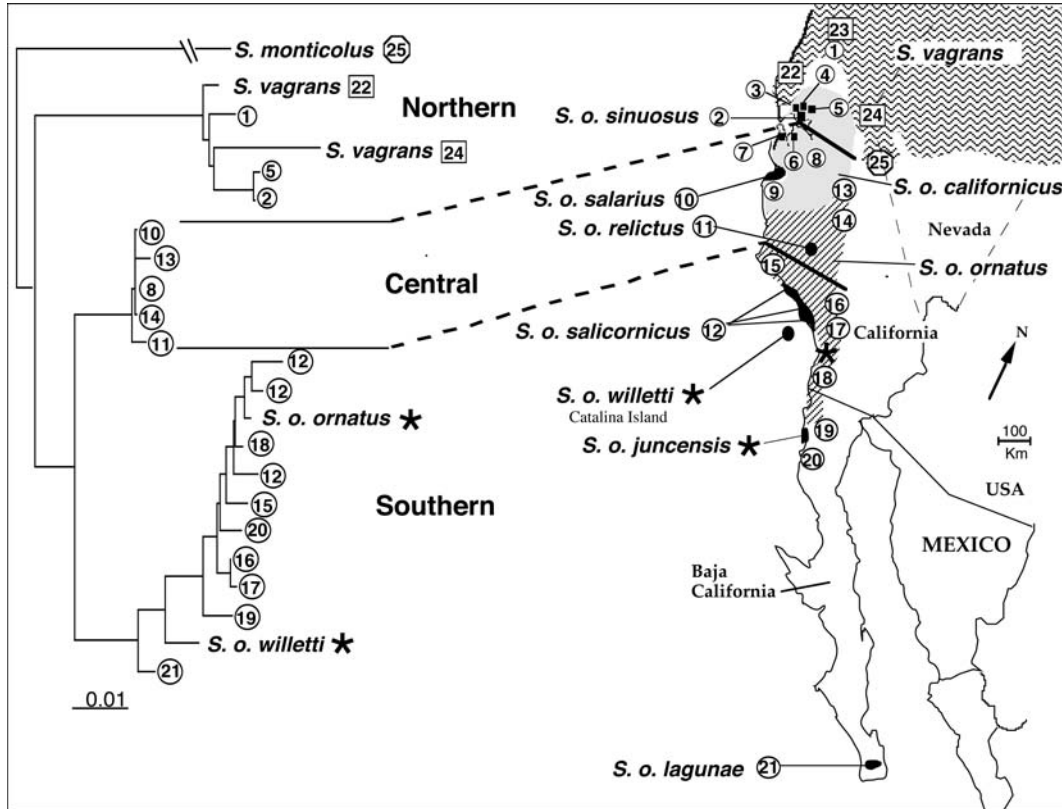
FIGURE 3. Map of the southwestern US and northwestern Mexico showing locations of populations sampled in Maldonado et al. (2004). Distribution of nine subspecies of ornate shrew (*Sorex ornatus*) is indicated. Thick lines indicate subdivisions based on genetic analyses (neighbor-joining tree based on average sequence divergence between populations, from Maldonado et al. 2001). Asterisks mark populations not sampled for morphometric study. Boxes indicate populations of wandering shrews (*S. vagrans*) and circles indicate populations of ornate shrews. The montane shrew population used as an outgroup is indicated with an octagon.

between them is only 0.009, and they share the same cytochrome *b* haplotypes. In contrast, the distance between Salinas and Grizzly Island and Rush Ranch is 150 km smaller, but their average allozyme distance and sequence divergence values are more than ten times greater (0.059 and 0.054, respectively). Considering genetic differentiation between marsh populations and their closest sampled inland relatives, there is a relatively large sequence divergence (1.2–1.3%) from Rush Ranch-Grizzly Island (*Sorex ornatus*) to Bodega Bay (the closest sampled population being wandering shrews.

Evolutionary history and biogeography

As suggested by low rates of gene flow, shrews are poor dispersers, and the imprint of past events may be long retained in present day populations. Clades have a high genetic divergence (4.2–4.9% cytochrome *b* sequence

divergence), suggesting a relatively long evolutionary independence from one another. Based on molecular data, populations in the northern clade diverged from the central and southern populations >1 MYA, and genetically are more similar to neighboring populations of wandering shrews. Northern clade ornate shrew haplotypes from Grizzly Island, Rush Ranch, and Dye Creek localities are grouped with those attributable to wandering shrews from Bodega Bay (population 22), Mt. Shasta (population 23), and the Sweetwater Mountains (population 24). Since ornate shrews could not be genetically differentiated from the wandering shrew in the northern region, Maldonado et al (2001) hypothesized that northern populations of the ornate shrew may be unique lowland and coastal forms of the wandering shrew that have converged independently on the morphology of southern and central California ornate shrews. However, by analyzing skull

coastal saltmarshes along San Francisco Bay, southern California, and Monterey Bay. These latter two populations have been, respectively, described as *R. m. limicola* (Von Bloeker 1932) and *R. m. distichlis* (Von Bloeker 1937), based on darker pelage coloration than the more widespread grassland form (*R. m. longicaudus*) that occurs in the adjacent upland habitats. Fisler (1965) found a similar tendency to dark pelage coloration in western harvest mice inhabiting the San Francisco Bay area; however, Pearson (1951) could not find differences between *R. m. distichlis* and *R. m. longicaudus*. Furthermore, Collins and George (1990) found no significant morphological or allozymic evidence to support the continued recognition of *R. m. limicola* in southern California. The results of their genetic and phenetic analysis suggest that within populations of *R. megalotis* in southern California no historical geographic units exist. Rather, broad phenetic overlap occurs among the samples suggesting that only gradual, small-scale phenetic change occurs among *R. megalotis* populations in mainland southern California.

Evolutionary history, and biogeography

Originally, the specific status of salt marsh harvest mouse was based on its sympatry with its then putative sister taxa, the western harvest mouse (Hooper 1952, Fisler 1965, Shellhammer 1967). However, analyses of karyotypic and allozymic data presented by Hood et al. (1984) and Nelson et al. (1984) suggested that salt marsh harvest mouse was both most closely related to the allopatric plains harvest mouse (*Reithrodontomys montanus*) from which it is distinct at the species level. This was supported by cytochrome *b* sequence data (Bell et al. 2001), where genetic distances separating salt marsh harvest mouse from plains harvest mouse and western harvest mouse ranged from 13.50% and 14.75%, respectively. These values are greater than those for other currently recognized biological species such as Sumichrast's harvest mouse (*Reithrodontomys sumichrasti*) and the western harvest mouse (9.79%) and Sumichrast's harvest mouse and Zacatecas harvest mouse (*Reithrodontomys zacatecae*) (8.55%). Using a sequence divergence value of 3.5% per million years, Bell et al. (2001) estimated that the salt marsh harvest mouse and plains harvest mouse diverged from a common ancestor 3.9 ± 0.7 MYA. This places the divergence of the salt marsh harvest mouse as far more ancient than the formation of saltmarshes around San Francisco Bay (Malamud-Roam, *this volume*).

Perhaps the salt marsh harvest mouse diverged from the plains harvest mouse when the Monterey Bay was a drainage basin. Approximately 5 MYA large areas in central California were covered by sea (Wahrhaftig and Birman 1965). Throughout much of the period that followed, the San Joaquin Valley was a wide seaway rather than the present day continental river valley and suitable mesic habitats for harvest mice were restricted to the margin of this seaway. One of the most profound barriers developed at the point were the present day central valley drains into the Pacific Ocean near Monterey Bay and where the largest marine canyon of the Pacific coast of North America is found (Yanev 1980). Throughout most of the Pliocene-Pleistocene, this area was a vast embayment that likely prevented dispersal for small vertebrates (Peabody and Savage 1958). A number of studies (Barrowclough et al. 1999, Rodriguez-Robles et al. 1999, Conroy and Cook 2000, Bronikowski and Arnold 2001, Maldonado et al. 2001, Rodriguez-Robles et al. 2001) have identified a deep phylogeographic break in northern California, often at or north of San Francisco Bay. In the case of the salt marsh harvest mouse its distribution is exclusively in the San Francisco Bay area and occurs in sympatry with the western harvest mouse also endemic to the tidal marshes of the San Francisco Bay area. Perhaps when the barriers to dispersal disappeared, the less specialized western harvest mouse expanded its range into this area and could have excluded the expansion of the upland adapted plains harvest mouse. The salt marsh harvest mouse became restricted to tidal marsh habitat where it outcompetes the western harvest mouse. Studies by Fisler (1965) suggest that the salt marsh harvest mouse is better adapted to tidal marshes than the western harvest mouse. For example, the salt marsh harvest mouse can drink and tolerate salt water, has a water-repellant pelage, and is a better swimmer. Possibly due to convergent evolution from occupying similar habitats, the western harvest mouse and the salt marsh harvest mouse are more similar morphologically than the salt marsh harvest mouse is to the plains harvest mouse.

Intraspecific differentiation in the salt marsh harvest mouse appears to be very low, although data exist for only two individuals: one sample from Tolay Creek representing the northern subspecies (*R. r. halicoetes*), and one sample from Newark representing the southern subspecies (*R. r. raviventris*; Bell et al. 2001). The Tamura-Nei genetic distance values were low (0.0018) suggesting at lack of differentiation at the intraspecific level.

SYNTHESIS

MOLECULES VERSUS MORPHOLOGY

Historically, the study of geographic variation in phenotype and morphology has been central to understanding evolutionary processes, under the assumption that morphology reflected an underlying genetic structure (Mayr 1942, Mayr 1963). In some cases, e.g., Swamp Sparrows, Clapper Rails, ornate shrews, and water snakes, morphological differentiation and differentiation at neutral loci are not concordant. In other cases, morphology and genetics only coincide some of the time. Within the Seaside Sparrows and sharp-tailed sparrows, the most prominent genetic finding is that each is divided into two widely divergent groups based on mtDNA (Avise and Nelson 1989, Rising and Avise 1993). In sharp-tailed sparrows, behavioral, and to some extent morphological, evidence corroborated this split (Greenlaw 1993), but in Seaside Sparrows, the two groups were not recognized before the genetic work was done, and no known morphological or behavioral differences separate the groups. Cases exist, however, such as in the Savannah Sparrows, sharp-tailed sparrows, and salt marsh harvest mice, where morphologically endemic species or subspecies were identified and their dissimilarities confirmed with surprisingly large amounts of genetic divergence at neutral loci.

Several explanations can account for the lack of concordance between molecules and morphology. As has been noted in ornate shrews (Maldonado et al. 2001, 2004) and Song Sparrows (Smith 1993), phenotypic differences in integument coloration, body size, and other morphological characters may be primarily developmental rather than genetic in origin. Alternately, evolutionary patterns at neutral loci and loci under selection may differ. With selection on quantitative trait loci, phenotype could diverge rapidly, while neutral loci diverge at a rate proportional to the effective population size. This lineage sorting may be incomplete, even in well-established species (see Funk and Omland 2003 for a review).

This is an area where few studies have explored and quantified those differences. We are still trying to understand with common garden and reciprocal-transplant experiments whether the differences between upland and tidal-marsh endemics are genetically based. Indications in Clapper Rails and Swamp Sparrows are that differences in phenotypic traits such as interorbital bridge widths and plumage are primarily genetically based (Olson 1997; R. Greenberg, unpubl. data), and in Song Sparrows, that morphology may be somewhat plastic (Smith 1993). Further studies with quantitative trait loci and on functional genes are promising avenues for future research in determining the strength of selection on tidal-marsh endemics.

ORIGIN OF TIDAL-MARSH TAXA

Genetic data combined with molecular-clock calibrations provide an estimate of the timing of divergence of taxa that occupy tidal marshes. Because these data only pertain to the accumulation of mutations in neutral markers in populations, they provide an estimate of the amount of time these taxa have experienced a unique evolutionary trajectory. These estimates do not provide insight into how long populations may have occupied tidal marshes, particularly if these populations have gone through ancient or recent periods of genetic connection with non-tidal marsh populations. Nor do they provide an estimate of how long divergent taxa may have been restricted to tidal marshes. But as a starting point, they provide a picture of the timing of the initiation of genetic divergence.

With the exception of terrapins and salt marsh harvest mice, the estimates of past population coalescence fall within the time period defined by the late Pleistocene to possibly as late as the Holocene. Within this time frame, a majority of taxa analyzed show divergence in the late Pleistocene with a smaller number dating back to the late Pliocene or early Pleistocene. Presumably, a pattern of Pleistocene colonization and differentiation characterizes other temperate zone habitats as well. However, we have been unable to find a similar habitat-based cross-taxa analysis to this one.

The ages of these splits vary by taxon within every region. For example, in northeastern North America, the Saltmarsh Sharp-tailed Sparrow is estimated to have been independent from the inland forms of the Nelson's Sharp-tailed Sparrow for 600,000 yr (Rising and Avise 1993), while the saltmarsh form of the Swamp Sparrow evolved from upland relatives <40,000 yr ago (Greenberg et al. 1998). On the West Coast, saltmarsh forms of the Song Sparrow and ornate shrew are barely distinguishable at genetic loci from neighboring upland forms (Maldonado et al. 2001, Chan and Arcese 2002), while the salt marsh harvest mouse living in the same marshes split from its closest upland relative nearly 4 MYA (Bell et al. 2001). On the Gulf Coast, Clapper Rails of saltmarshes widely share mtDNA haplotypes with King Rails of freshwater marshes (Avise and Zink

1988; R. C. Fleischer, unpubl. data), but Seaside Sparrows probably diverged from an upland ancestor 1.5–2 MYA (Rising and Avise 1993), and diamondback terrapins probably did so at 7–11 MYA (Lamb and Osentoski 1997). The timing of those divergences ranges from Holocene to early Pliocene to late Miocene, and suggest a complex history of multiple invasions and differentiations in saltmarshes.

One factor that may have aided differentiation of tidal-marsh taxa is the restricted dispersal indicated by behavioral and ecological studies in many species. In many cases ecological studies have noted higher philopatry and reduced dispersal of tidal-marsh endemics compared with closely related upland species. In ornate shrews, limits to physiological tolerance of inhospitable habitats has been noted (McNab 1991, Maldonado et al. 2001). In Savannah Sparrows, most of the tidal-marsh forms are non-migratory, compared with migratory upland forms. In Song Sparrows, ecological studies suggested that drift may have played a large role in their differentiation, with tidal-marsh forms showing some of the shortest dispersal distances recorded for a Song Sparrow as well as shortened wings (Marshall 1948a).

Low dispersal, combined with the resource-rich, homogenous landscape of the tidal-marsh habitat, may reduce gene flow to other habitats and increase local adaptation. In Red-winged Blackbirds (*Agelaius phoeniceus*), for example, genetic distance as measured from allozymes between populations in Sacramento and San Francisco bays (214 km apart) was 10 times as great as the genetic distance between Florida and Oregon (Gavin et al. 1991). Gavin et al. (1991) hypothesized that philopatry and non-migratory behavior may have caused this differentiation and discussed the possibility that the brackish environment in which they live may enforce a selective regime that could reduce immigration or emigration to other habitats.

The geological history of the tidal marshes may provide some insight into the evolutionary origin of tidal-marsh taxa. For many of the taxa, the last Pleistocene glacial maximum was hypothesized to play a role in their diversification, and in some cases, such as the Swamp Sparrow, the molecular evidence supports this hypothesis (Greenberg et al. 1998). In other examples such as the salt marsh harvest mouse and diamondback terrapin, diversification predates the Pleistocene. Although the last glacial maxima may not have played a large role in the speciation of tidal-marsh endemics, in the Gulf and Atlantic coasts, genes have recorded the geological history as revealed by comparative phylogeography of Seaside Sparrows and

diamondback terrapins (Rising and Avise 1993, Lamb and Osentoski 1997).

Furthermore, perhaps differences in the stability of the habitat over time may provide clues as to how quickly phenotypic differentiation can occur. For the most part, though, a concordance is lacking between geology and differentiation with respect to the origination of tidal-marsh taxa. For example, in the case of the salt marsh harvest mouse, the amount of divergence from its presumed sister taxon greatly exceeds the age of its current habitat. The entire range of the salt marsh harvest mouse is now restricted to tidal-marsh habitats surrounding San Francisco Bay; however, those habitats did not exist as such 10,000 yr ago, and have gone through periodic inundations as sea levels rose and receded during glaciations. It remains a challenge to match our new understanding of the evolutionary dynamics of tidal-marsh vertebrates with geologic reconstructions of coastal-marsh history.

CONSERVATION GENETICS

The uniqueness of tidal marsh forms, in combination with the rapid destruction and development of tidal marsh habitat in North America, has led to efforts for their protection and proper management. The preponderance of taxa that show significant divergence in morphology and life history with difference in molecular makers demands that we assess the definition of important conservation taxonomic units when approaching tidal-marsh conservation.

The U.S. Endangered Species Act of 1973 together with the Distinct Population Segment (DPS) amendment in 1978 protects distinct species, subspecies, and populations. Since designation provides large financial resources as well as immediate protection from hunting, habitat exploitation, and other anthropogenic impacts that threaten population viability, much debate surrounds the criteria used to designate units of management and conservation (O'Brien and Mayr 1991). See also Fraser and Bernatchez (2001) for a discussion of conservation units.

Several definitions for conservation units (O'Brien and Mayr 1991, Moritz 1994) attempt to incorporate meaningful criteria to identify groups of populations with distinct evolutionary potential. Most of these are based on phylogenetic distinctness (subspecies—Avise and Ball 1990, O'Brien and Mayr 1991, Ball and Avise 1992; evolutionarily significant units (ESUs)—Ryder 1986, Moritz 1994, Moritz et al. 1995). This is because phylogenetic partitioning results from accumulation of differences due to the lack of gene flow (O'Brien and Mayr 1991).

However, the emphasis on genetic criteria has been criticized, and an approach that incorporates adaptive differences based on ecological and genetic exchangeability is advocated by Crandall et al. (2000).

Although results from genetic studies led directly to the designation of species status for the Nelson's and Saltmarsh sharp-tailed sparrows, in Savannah and Seaside sparrows, large genetic divergence has not yet been accompanied by specific status, although few would question their recognition as ESUs. In Clapper Rails, genetic studies alone provided little basis for the recognition of a taxon as a distinct species, or even as ESUs; however, their taxonomic status has not been modified because their distinctness remains defendable based on morphological and habitat differences. The same is true for the Coastal Plain Swamp Sparrow, which is morphologically and ecologically well-differentiated from freshwater subspecies, but shows no reciprocal monophyly of mtDNA sequences nor significant allele frequency differences. Since the criteria used to define taxonomic and conservation units are still being debated and because many tidal marsh taxa are threatened, their status will likely remain contentious as well.

Besides contributing to defining conservation units, molecular studies can improve genetic management of endemic tidal marsh taxa. The naturally fragmented and linear nature of the tidal-marsh habitat has the possibility of increasing intraspecific genetic structure, complicating proper management and restoration programs. For example in Seaside Sparrows, being aware of the phylogeographic structure underlying the species would have better informed attempts to save the Dusky Seaside Sparrow. Populations with low heterozygosity, as indicated by molecular studies, such as those of the Light-footed Clapper Rail, should be studied carefully for indications of inbreeding depression (Keller and Waller 2002), and if in danger of extinction may benefit from management efforts that increase gene flow and genetic variability, and prevent genetic erosion.

However, increasing gene flow and variability may also be harmful to the integrity of tidal-marsh endemics. Anthropogenic impacts, such as habitat disturbance and climatic change have the potential to change selective forces in the marshes over short-term ecological timescales and may increase introgression (Takekawa et al. chapter 11, *this volume*). Conant (1963) speculated that interbreeding between the water snakes (*Nerodia sipedon williamengelsi* and *N. fasciata*) represented introgressive hybridization resulting from habitat alteration. In San Francisco Bay, the one genetically distinct subspecies of Song Sparrow (*Melospiza melodia pusillula*), may be in danger of introgression from the upland subspecies nearby if salinity changes in the tidal saltmarshes due to urbanization.

Furthermore, natural hybridization may reduce the range of the strict saltmarsh endemics, increasing priority for non-introgressed populations. Although the zone of hybridization appears to be geographically stable in Sharp-tailed Sparrows in southern Maine (Montagna 1940, 1942; Rising and Avise 1993, Shriver 2002), and the front where hybridization can occur is narrow due to the linear nature of tidal marsh habitat, there is recognizable introgression of *Ammodramus nelsoni subvirgatus* genes into *A. caudacutus caudacutus* populations as far south as Parker River, Maine (Shriver 2002). Shriver (2002) points out that this introgression reduces the known range of pure *A. c. caudacutus*, and perhaps should increase conservation priority in the restricted range of this species.

As habitat destruction and global climatic change continue to reduce and fragment tidal marsh habitat and their containing populations, the use of molecular markers will be even more essential to implement for conservation.

FUTURE STUDIES

The use of molecular approaches to study tidal-marsh taxa has just begun. Many of the DNA sequence and RFLP studies described in this review are from the mitochondrial genome, which has advantages as well as disadvantages. MtDNA has a high mutation rate, maternal mode of transmission, and lack of recombination (Avise 2000). A high mutation rate is particularly valuable for detecting variation among closely related taxa and a lack of recombination facilitates phylogenetic reconstruction. However, the entire mitochondrial genome is a single locus resulting in one part of the mitochondrial genome influencing all others through linkage, preventing any site from being independent. Furthermore, the maternal inheritance of mtDNA can produce incongruence between population history and gene history when sex differences occur in dispersal or fitness (Ballard and Whitlock 2004). In order to better resolve the population history of endemic tidal-marsh taxa many of these studies need to be extended to nuclear loci. Furthermore there are also more powerful analytical tools for reconstructing population history, such as coalescent approaches that have not been widely applied to tidal-marsh endemics.

Coalescent approaches are particularly appropriate techniques for understanding the

evolutionary history of tidal-marsh taxa. The coalescent is a stochastic process that can be used to model past population demographic history and provides a statistical framework for data analysis (see review in Rosenberg and Nordborg 2002). Analysis of genetic polymorphism data utilizing coalescent approaches enables us to address competing demographic and biogeographic hypotheses; it can be used within a hypothesis testing framework to test alternate scenarios for colonization and subsequent differentiation. Hypotheses based on geological reconstruction can be tested with genetic data providing a better understanding of the process of differentiation in endemic taxa. Issues that can be more readily addressed with coalescent approaches are: The number of colonization events, the need for a bottleneck for differentiation, and the lack of subsequent gene flow. Furthermore, combined with likelihood methods, coalescent approaches allow not just hypothesis testing, but parameter estimation; the effective population size, extent of gene flow, and timing of divergence.

The tidal-marsh habitat provides a unique field laboratory to study intraspecific spatial genetic structure and metapopulation dynamics. Because tidal saltmarshes are discrete and often patchily distributed, they are ideal places to study the extent and amount of gene flow between patches. The relative homogeneity of the habitat lends itself to straightforward quantification of population sizes for comparison to genetic estimates of effective population size, providing many of the necessary parameters for population genetic models. In addition, their discrete and patchy distribution may act to reduce the ability of genetically based divergence to evolve in local tidal-marsh populations. This distribution results in a large edge effect that may reduce the isolation of tidal marsh and other habitats. Furthermore, as documented by Malamud Roam et al. (*this volume*), tidal marshes have been highly unstable in their location and extension throughout recent geological history. The ice sheets covered high-latitude tidal marshes and many of the largest areas of estuarine tidal marsh located at mid-latitudes are a result of flooding from the sea-level rise associated with the melting of Pleistocene glaciers. However, lower sea levels during the glacial periods may have exposed more coastal plain and increased tidal marshes in some areas. It will also be interesting to document genetic changes in tidal-marsh organisms in response to potential changes in saltmarsh distributions that may occur with changes in sea level due to global warming.

Furthermore, several endemic taxa have yet to be examined using molecular approaches, such as the California Black Rail (*Laterallus jamaicensis coturniculus*), and subspecies of Marsh Wren (*Cistothorus palustris*), Common Yellowthroat (*Geothlypis trichas*), California vole (*Microtus californicus*), meadow vole (*Microtus pennsylvanicus*), and masked shrew (*Sorex cinereus*).

The possibility of studies of selection on functional genes is particularly exciting. For example, it may prove important to test the hypothesis set forth by Gavin et al (1991) that the large amounts of genetic differentiation of Red-winged Blackbirds from the San Francisco Bay and the Salton Sea may be the result of physiological adaptations to saltmarsh environments that prevent or reduce interchange with populations living in upland or freshwater environments. Furthermore, this hypothesis could be tested by examining physiological tolerance for salt water and genetic evidence for gene flow among populations of various vertebrates found in salt and freshwater environments.

The study of tidal-marsh taxa is a special opportunity as part of the larger paradigm of geographic variation. Here we have the opportunity to synthesize how different vertebrate groups, respond to the same environmental pressures, such as salinity (Goldstein, *this volume*), periodic flooding (Reinert, *this volume*), substrate color, and available food (Grenier and Greenberg, *this volume*) with a variety of different ages and extent of gene flow. Molecular approaches, combined with morphological, ecological and behavioral studies, will continue to improve our understanding and aid conservation of tidal marsh vertebrates in the future.

ACKNOWLEDGMENTS

ADAPTATION TO TIDAL MARSHES

Salt marsh harvest mouse (*Reithrodontomys raviventris*)
Drawing by Julie Zickefoose