GEOGRAPHIC PATTERNS OF MITOCHONDRIAL-DNA VARIATION IN COLLARED PECCARIES

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We investigated variation in mitochondrial DNA (mtDNA) of 84 collared peccaries (*Tayassu tajacu*) from Arizona and four peccaries from Texas using 12 restriction enzymes to determine the geographic pattern of variation in this social, relatively recent immigrant to Arizona. Three mtDNA haplotypes were present in the sample from Arizona, and sequence divergence estimates among these ranged from 0.2 to 0.8%. The frequencies of these three haplotypes differed significantly across geographic regions, indicating some partitioning of mtDNA haplotypes. Analysis of these data suggests that Arizona was colonized by at least three mtDNA lineages, most likely originating in Mexico. We hypothesize that founding events at the edge of the peccary's expanding range led to the initial partitioning of haplotypes observed in samples from Arizona.

Key words: *Tayassu tajacu,* collared peccary, Arizona, mitochondrial DNA

Geographic patterns of genetic variation are the result of a population's evolutionary history and how that history has affected past and current levels of gene flow (Avise, 1989; Avise et al. 1987; Slatkin, 1987). Genetic differentiation can arise through the action of mutation, selection, and drift on isolated populations and through colonization events by non-representative subsets of parental populations (Slatkin, 1987). Theoretical models have suggested that colonization will contribute most to genetic differentiation when founding groups comprise genetically related individuals or kin-groups (Hedrick and Levin, 1984; Whitlock and McCauley, 1990). Kin-founding events would be more probable in social animals and have been argued to be a major mechanism leading to genetic differentiation of various social primates (Chepko-Sade and Sade, 1979; Cheverud and Dow, 1985; Cheverud et al., 1978; Fix, 1975; Neel and Ward, 1972; Smouse et al., 1981). Once geographic genetic differentiation was established, behaviors such as territoriality and philopatry could act to maintain that differentiation by limiting gene flow between colonies and the parental population.

We studied the patterns of geographic variation in mitochondrial DNA (mtDNA) of collared peccaries (*Tayassu tajacu*) from Arizona because they are relatively recent immigrants into Arizona and exhibit many of those characteristics which could lead to genetic differentiation through colonization events. In the southwestern United States, collared peccaries live in stable social groups that defend territories from adjacent herds (Bissonette, 1982; Byers and Bekoff, 1981; Day, 1986). These herds can contain males and females of all ages and have been hypothesized to be an extended family of genetically related individuals (Byers and Bekoff, 1981). Colonization of unoccupied area by a herd could, therefore, represent a kin-founding event. Although the amount of movement by individuals between herds varies, several studies have shown exchanges to be relatively rare (Bissonette, 1982; Byers and Bekoff, 1981; Day, 1986; Schweinsburg, 1971). When dispersal does occur, it appears to be into adjacent herds

and often is male biased (Day, 1986). These traits could lead to relatively low levels of gene flow between subpopulations of peccaries, especially in maternally inherited mitochondrial genes.

Collared peccaries entered the United States from Mesoamerica by two separate routes; one along the Pacific coast into Arizona and the other along the Atlantic coast into Texas (Day, 1986; Sowls, 1984). These two branches of distribution of peccaries separate in southcentral Mexico and remain geographically disjunct northward to Texas and Arizona (Hall, 1981). Comparison of mtDNA divergence between peccaries from Texas and Arizona, therefore, allows estimation of the amount of divergence in populations separated for hundreds of thousands if not millions of years. In contrast, peccaries are believed to have entered Arizona only in the past few thousand years and have been expanding their range northward in historic times (Day, 1986; Sowls, 1984). In the present study, we examined the patterns of geographic variation in mtDNA of peccaries within Arizona and estimated the amount of sequence divergence that has occurred between populations of peccaries in Arizona and Texas.

Materials and Methods

Samples of muscle or liver were obtained from 84 hunter-killed peccaries from the following sites in Arizona: Chiricahua Mountains, Cochise Co. ($n = 9$); Wilcox, Cochise Co. ($n = 2$); Huachuca Mountains, Cochise Co. ($n = 22$); Baboquivari Mountains, Pima Co. ($n = 20$); Aravaipa Valley, Graham Co. ($n = 5$); San Carlos Indian Reservation, Gila and Graham counties ($n = 5$); Tonto Basin, Gila Co. ($n = 7$); Verde Valley, Yavapai Co. ($n = 8$); Bartlett Lake, Maricopa Co. ($n = 4$); Wickenburg, Yavapai Co. ($n = 2$). In addition, samples from four peccaries from Zavala Co. in southcentral Texas were obtained from the Texas Cooperative Wildlife Collection, Texas A&M University. Total genomic DNA was isolated from samples by phenol-chloroform (Maniatis et al., 1982) or salt-chloroform extraction (Mullenbach et al., 1989). We developed the mtDNA probe for peccaries by first enriching the mitochondrial fraction of total genomic DNA through cell lysis followed by centrifugation. The fraction containing mtDNA was then digested with *Eco*RI and separated into fragments in a low-melting-point agarose gel by electrophoresis. The two putative mtDNA fragments were isolated and combined. These fragments hybridized to a known mouse-mtDNA probe obtained from D. A. Clayton.

All samples were digested with 12 restriction enzymes (*Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Hin*fI, *Kpn*I, *Msp*I, *Pst*I, *Rsa*I, and *Taq*I) for 4 h followed by electrophoresis at 25 volts on either 0.7%-agarose gels (for restriction enzymes with hexanucleotide-recognition sites) or 1.5%-agarose gels (for restriction enzymes with tetranucleotide-recognition sites). Fragments of DNA were transferred from gels to nylon membranes (Southern, 1975), probed with a radiolabeled mitochondrial probe for collared peccary, and the mtDNA fragments were visualized by autoradiography. All samples were digested and separated into fragments by electrophoresis repeatedly with digested and undigested controls to verify the presence or absence of restriction sites.

Sizes of mtDNA fragments were estimated by comparison with DNA standards (Lambda bacteriophage DNA digested with *Hin*dIII). Estimates of sequence divergence were calculated using equation 21 and fig. 1 from Nei and Li (1979). Final values of sequence divergence were computed as the weighted mean of values from restriction enzymes with tetranucleotide- and hexanucleotide-recognition sites. The significance of geographic heterogeneity in distribution of haplotypes was tested using chi-square-contingency-table analysis. Because small samples resulted in >20% of the contingency table cells having expected values of five or less, we used the Monte Carlo simulation of Roff and Bentzen (1989) to verify the significance of our chi-square analysis.

Results

Total size of mtDNA of peccaries was estimated at ca. 16,400 ± 200 base pairs based on fragments produced by digestion with *Bst*EII, *Eco*RI, *Eco*RV, and *Kpn*I. The 12 restriction enzymes used in this study surveyed 63–68 restriction sites, ca. 300 base pairs, or ca. 2% of the total mtDNA molecule.

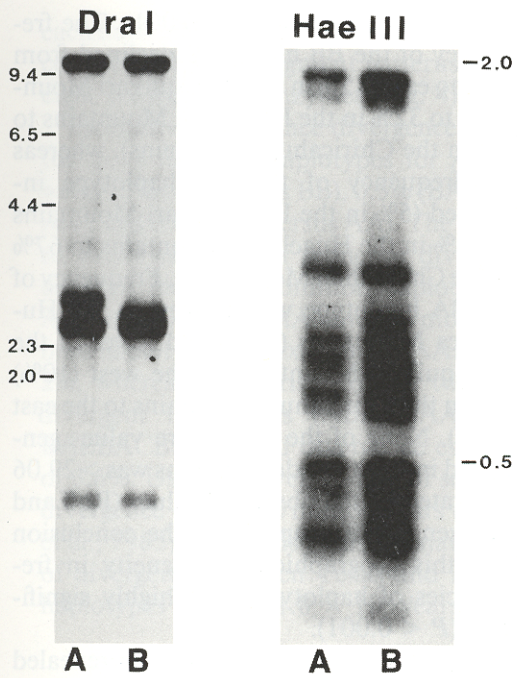

FIG. 1.—Patterns of mitochondrial-DNA restriction fragments of peccaries from Arizona when digested with the restriction enzymes *Dra*I and *Hae*III. Two patterns were observed for each enzyme (labelled A and B). Standards for DNA of known size (given in kilobases) are indicated along the margin of the pattern of fragments.

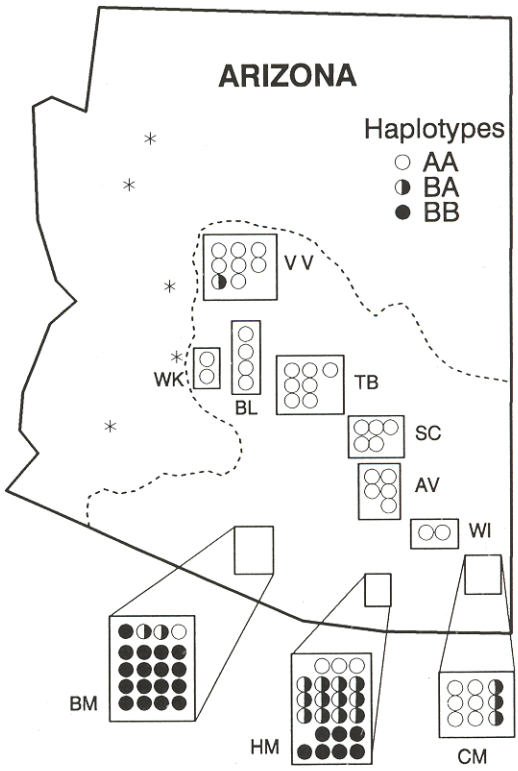

FIG. 2.—The geographic distribution of the three mtDNA haplotypes (AA, BA, and BB) found in samples of 84 peccaries from Arizona. Areas sampled are designated as follows: VV, Verde Valley; WK, Wickenburg; BL, Bartlett Lake; TB, Tonto Basin; SC, San Carlos Indian Reservation; AV, Aravaipa Valley; WI, Wilcox; CM, Chiricahua Mountains; HM, Huachuca Mountains; BM, Baboquivari Mountains. Asterisks mark locations where the Arizona Game and Fish Department transplanted peccaries originally captured in southern areas of the state. The dashed line approximates the present natural distribution of the collared peccary in Arizona.

Of the 12 restriction enzymes surveyed, only *Dra*I and *Hae*III yielded polymorphic patterns among the 84 peccaries from Arizona (Fig. 1). Digestion with *Dra*I yielded two patterns, designated A and B. One of the fragments in haplotype B was ca. 0.2 kilobases smaller than the similar-sized fragment in haplotype A and co-migrated with one of the other fragments. Digestion with *Hae*III also resulted in two patterns (designated A and B), which differed by one fragment. Three haplotypes (AA, BA, and BB, with the first letter representing patterns from *Dra*I and the second patterns from *Hae*III) were observed in the 84 animals from Arizona.

The distribution of mtDNA haplotypes in Arizona showed significant geographic partitioning (Fig. 2). The AA haplotype was, with one exception, the exclusive haplotype in samples from central and northern Arizona. Although the AA haplotype was present in the three southern populations, comparison of frequencies of haplotypes in the Baboqivari Mountains (n = 20), the Huachuca Mountains (n = 22), and the Chiricahua Mountains (n = 9), showed a significant deviation from a random distribution

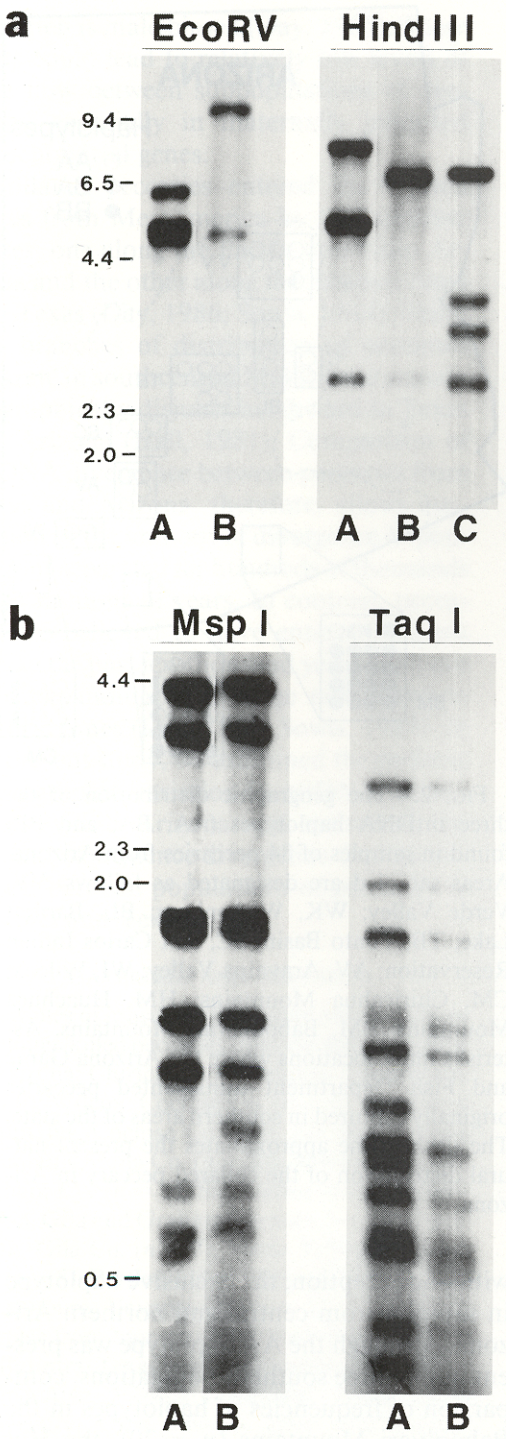

FIG. 3.—Patterns of mitochondrial-DNA restriction fragments obtained for collared peccaries from Arizona and Texas (a) with the restriction enzymes *Eco*RV and *Hin*dIII and (b) with *Msp*I and *Taq*I. In each panel, patterns labelled A represent peccaries from Arizona, and patterns labelled B and C represent peccaries from Texas. Standards for DNA of known size (given in kilobases) are indicated along the margins.

($\chi^2 = 29.06$, $d.f. = 4$, $P < 0.001$). The frequency of the BB haplotype declined from west to east (85% in the Baboquivari Mountains to 32% in the Huachuca Mountains to 0% in the Chiricahua Mountains), whereas the frequency of the AA haplotype increased (5% in the Baboquivari Mountains to 13% in the Huachuca Mountains to 67% in the Chiricahua Mountains). Frequency of the BA haplotype was greatest in the Huachuca Mountains (55%) and lower in the Baboquivari Mountains to the west (10%) and in the Chiricahua Mountains to the east (33%). None of the chi-square values generated in 1,000 randomizations was >29.06 generated by the observed values (Roff and Bentzen, 1989), supporting the conclusion that this geographic heterogeneity in frequencies of haplotypes was highly significant ($P < 0.001$).

Six of the 12 restriction enzymes revealed differences between the restriction patterns of samples from Arizona and Texas. Enzymes *Bst*EII and *Eco*RI yielded two mtDNA fragments in samples from Arizona, but there were no restriction sites for these enzymes in samples from Texas. The other four restriction enzymes yielded patterns for samples from Arizona and Texas that differed in fragment number or size (Fig. 3). Within the four samples from Texas, one of the 12 restriction enzymes yielded a polymorphic pattern. Digestion with *Hin*dIII resulted in either three or four fragments due to a difference (gain or loss) of one restriction site (Fig. 3). A total of five haplotypes could be distinguished in peccaries based on restriction-site differences among and within the samples from Arizona and Texas. A hand-drawn parsimony network was constructed for these five haplotypes, with

each haplotype designated by an eight-letter code representing fragment patterns for the eight polymorphic enzymes (Fig. 4).

Estimates of sequence divergence among the three mtDNA haplotypes for Arizona were 0.2% (BA versus BB), 0.5% (BA versus AA), and 0.8% (BB versus AA). The weighted mean estimate of sequence divergence between the most common haplotype for Arizona (AAAAAAAA) and the most common haplotype for Texas (BABBAABB) was 2%, whereas comparison of the two most divergent haplotypes (ABAABAAA and BABBACBB) yielded divergence estimates of 3.6%. Using an average rate of sequence divergence of ca. 2%/million years (Wilson et al., 1985), this leads to an estimated time of divergence between Texas and Arizona haplotypes of ca. 1–1.8 × 10^6 years ago.

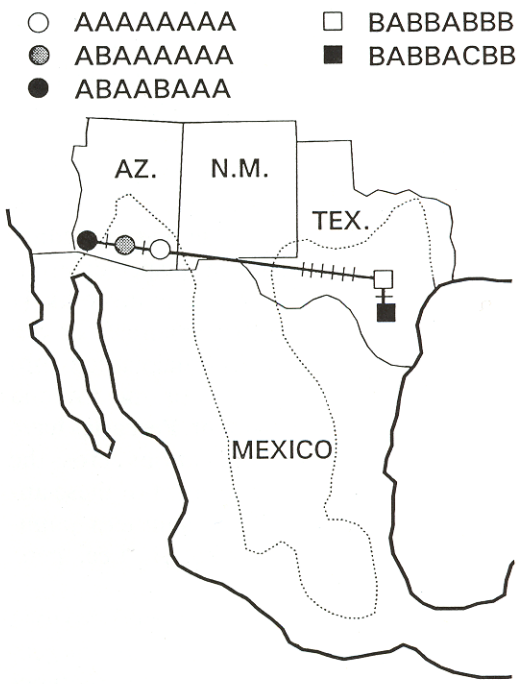

FIG. 4.—A hand-drawn parsimony network for the five mtDNA haplotypes found in a survey of collared peccaries from Texas (n = 4) and Arizona (n = 84). Each letter of the haplotype represents the pattern of restriction fragments generated by restriction enzymes *Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Msp*I, and *Taq*I, respectively. Each hatch mark on the line connecting haplotypes indicates one restriction site difference. All samples from Texas were taken in Zavala Co. The dotted line approximates the present natural distribution of the collared peccary in North and Central America.

DISCUSSION

The estimates of sequence divergence among the three mtDNA haplotypes for Arizona were similar to intraspecific estimates in rodents (Rodentia—Avise et al., 1979; Avise et al., 1983), horses (George and Ryder, 1986), humans (Brown, 1980; Cann et al., 1987), fruit bats (*Artibeus jamaicensis*—Pumo et al., 1988), and deer (*Odocoileus*—Carr et al., 1986; Cronin, 1990). Although the number of haplotypes found in peccaries from Arizona was low compared to some rodent and avian species (Avise et al., 1979; Avise et al., 1983; Ball et al., 1988), it was comparable to the number found in other large mammals (Cronin et al., 1988, 1991; Wayne et al., 1990).

The estimates of sequence divergence among haplotypes for peccaries from Texas and Arizona were similar to those between two subspecies of mule deer (Carr et al., 1986). This amount of divergence is expected given that peccaries from Arizona and Texas have been geographically isolated for thousands of years and are morphologically different enough to be classified as different subspecies, *T. sonoriensis* and *T. t. angulatus*, respectively (Hall, 1981). These estimates place the separation of mtDNA lineages in Texas and Arizona in the late Pliocene or early Pleistocene. It has been argued that the genus *Tayassu* evolved either in Central (Simpson, 1980) or South America (Wetzel, 1977; Woodburne, 1969) during the late Pliocene. Therefore, the divergence of these haplotypes may have occurred fairly early in the evolution of this genus. The earliest fossil remains of *T. tajacu* in Central America date from the late Pleistocene (Woodburne, 1969), but there are no other fossil remains that indicate when the ancestral population divided to give rise to the populations in Texas and

Arizona. However, dates of mtDNA divergence reflect only the time of haplotype divergence and may not coincide with the time of population subdivision (Avise et al., 1987).

The 32 peccaries from northern and central Arizona shared the same haplotype with one exception. This single variant could represent a long-distance disperser from southern populations, but it is more likely that this animal was transplanted, or descended from individuals transplanted, into unoccupied northern areas by the Arizona Game and Fish Department. Peccaries have been released in various locations across the state since the 1950s, and most of these animals were obtained from southern populations (Fig. 2; Day, 1986; R. Lee, pers. comm.).

Because peccaries have entered Arizona in only the past few thousand years, the geographic pattern of frequencies of haplotypes in Arizona more likely resulted from colonization events rather than de novo mutations arising after the area was colonized. The three haplotypes in samples from southern Arizona may represent colonization by three different maternal lineages from Mexico. The nonrandom frequency distribution of haplotypes in the three sites in southern Arizona suggests either that maternal gene flow among these regions is restricted or that colonization by the BA and BB haplotypes has been so recent that there has not been time for them to spread into other regions. The absence of two of the mtDNA haplotypes in the central and northern regions of Arizona could have resulted from the founding of initial populations by animals sharing the AA mtDNA haplotype. One likely route of this expansion might have followed the Gila River drainage northwestward from the southeastern part of the state. The fact that the AA haplotype is most common in southeastern Arizona is consistent with this hypothesis.

The probability that founding groups would share the same haplotype would be greatest if group members were related. Field studies in Texas documented that new herds of peccaries were founded by subgroups that split away from a parental herd (Bissonette, 1982). Likewise, when a herd of peccaries in Arizona was experimentally removed from its territory, the vacant habitat created was subsequently colonized by a subgroup of adult males and females and their offspring from a neighboring herd (Supplee, 1981). Given the strong social bonds that exist among members of a herd, colonization by subgroups probably is typical of movement into unoccupied areas.

We hypothesize that founding events have led to the geographic partitioning of mtDNA haplotypes in Arizona. Although rapidly expanding populations have been hypothesized to show little geographic partitioning of mtDNA haplotypes (Avise, 1989; Avise et al., 1987), the present study suggests that significant geographic differentiation may occur in social species in which founding groups have a high probability of relatedness.

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