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Genetics

Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas

(restriction endonuclease mapping/interspecific gene flow/*Odocoileus* species/wildlife biology)

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ABSTRACT **Sympatric populations of white-tailed deer and mule deer (*Odocoileus virginianus* and *Odocoileus hemionus*, respectively) on a west Texas ranch share a common mitochondrial DNA restriction map genotype. Phylogenetic analysis indicates that this genotype is more characteristic of *O. virginianus* than of *O. hemionus*. The genotype of west Texas deer differs from that of *O. virginianus* from South Carolina by five mutational events (1.3% sequence divergence), whereas it differs from that of *O. hemionus* from California by 17 events (5.5% divergence). We suggest that interspecies hybridization has occurred, primarily between mule deer bucks and white-tailed deer does, with preferential absorption of hybrid offspring into the mule deer gene pool. Introgressive hybridization may be involved in ongoing displacement of mule deer by white-tailed deer in west Texas.**

Natural hybridization between white-tailed deer (*Odocoileus virginianus*) and mule deer or black-tailed deer (*Odocoileus hemionus*) has long been suspected (1, 2). In New Mexico and west Texas, white-tailed deer have expanded their range westward at the expense of mule deer for some 40 years, and it has been suggested that hybridization might contribute to this displacement. Captive-breeding studies indicate that interspecific crosses are possible. Although most such matings are sterile, both reciprocal crosses can produce viable offspring, and at least some of these F_1 progeny have been fertile in all backcross combinations (3, 4). This problem is of interest from both practical and theoretical considerations. Deer are among the most economically important wildlife species in the United States (5), and hybridization between species of ungulates or other large mammals appears to be rare in nature (6, 7). We are aware of only one published genetic study of natural hybridization among ungulates, a study of bison subspecies (8). Up to the present, the hypothesis of interspecies hybridization in deer has not been tested by quantitative genetic means.

Mitochondrial DNA (mtDNA) has been used extensively in studies of population biology in recent years (9–11). The entire molecule has been sequenced in four vertebrate species, as a result of which the positions and natures of the coding sequences, tRNA genes, and rRNA regions are known. The gene order is identical in the four species sequenced thus far (12–15). Because mtDNA is a separate genetic system, outside the nucleus, and seems to be strictly maternally inherited, it has been used as a probe of interspecies gene flow (16–21).

The two species of the genus *Odocoileus* are distributed throughout North America. Mule deer (including conspecific black-tailed deer of the Pacific Northwest) are primarily western animals, whereas white-tailed deer are more common in the central and eastern United States and in Central America. The two species are broadly sympatric over much of their range (22). They are distinguished morphologically by several criteria, including the form of the antlers and the size of the metatarsal gland. In white-tailed deer, the antlers each consist of a single unbranched main beam, whereas in mule deer the antlers are forked into two equal branches. In white-tailed deer, the metatarsal gland is small (about 2 cm long), whereas in mule deer the gland is typically more than 9 cm in length. As antlers are a secondary sex characteristic of adult bucks, the metatarsal gland is an important character in the identification of does and juveniles.

In studies of ecological relationships between these species in west Texas, one of us (L.H.B.) has noted an increased incidence in recent years of deer with intermediate metatarsal gland morphology and general appearance. Based on such observations, we conducted an extensive sampling of Texas deer, including sympatric populations of white-tailed and mule deer from the Longfellow Ranch in Pecos County and allopatric populations of both species from neighboring ranches and other sites in central and east Texas. In order to compare the genetics of deer from the putative hybrid zone with representative populations of either species as far removed from west Texas as possible, we also examined black-tailed deer from the Hopland Field Station in California and white-tailed deer from the Savannah River Plant in South Carolina. Both of these populations are well outside the zones of species overlap (22).

MATERIALS AND METHODS

Deer from the sympatric populations at Longfellow Ranch were assigned species identifications in the field by L.H.B. and his assistants. These identifications were based on several characters, including antler form (when present) and length of the metatarsal gland (Table 1). The distributions of the latter character in samples of both species from the area of sympatry were significantly different ($t = 25.4$, $P < 0.005$) and did not overlap in range. All species identifications from this locality included in this study are therefore considered unambiguous. Based on similar criteria, identifications of deer in the allopatric populations in Texas and elsewhere are also considered unambiguous.

mtDNA was purified to apparent homogeneity by standard means (23) and digested with 10 type-II restriction endonucleases, both singly and in pairwise combinations. The resulting fragments were then radioactively end-labeled (24), separated electrophoretically in agarose or polyacrylamide gels, baked onto filter papers, and autoradiographed. Restriction maps were constructed by comparison of the single and pairwise fragment patterns of all enzymes. The extent of sequence divergence among genotypes was estimated from equation 16 of Nei and Li (25).

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Abbreviation: mtDNA, mitochondrial DNA.

Table 1. Distributions of metatarsal gland lengths in sympatric deer populations at the Longfellow Ranch, Pecos County, TX

Species	*n*	Gland length, cm	
		Mean ± SD	Range
Mule deer	37	10.1 ± 1.4	7.5–13.0
White-tailed deer	13	2.0 ± 0.8	1.2–4.2

RESULTS

A total of 45 restriction sites were mapped for 10 endonucleases (Fig. 1). Twenty-one sites are invariant and 24 are variable. The variable sites define seven distinct restriction map genotypes. The maps were aligned with the complete sequence from bovine mtDNA (14), using three restriction sites that are highly conserved throughout vertebrate evolution (23). The size of the deer mtDNA is estimated as 16.6 kilobase pairs. We found no evidence of length variation among any of the mtDNA genotypes.

Four of the 13 deer populations examined showed more than one mtDNA genotype (Table 2). These were the deer at Longfellow Ranch (see below), mule deer from the Kimball Ranch, and white-tailed deer from the Granada Ranch and San Angelo, all in Texas. The deer from the Savannah River Plant, which include individuals from distinct populations that are known to vary allozymically (26), were all identical with respect to their mtDNA restriction map patterns for these 10 endonucleases. We found no evidence of intraindividual mtDNA sequence heterogeneity in any of our samples.

The minimum-length network connecting the seven genotypes in Fig. 1 was determined by a maximum-parsimony approach. Fig. 2 shows this network overlaid on a map of the localities from which the genotypes were first isolated. The genotypes differ by between 1 and 17 restriction site gain/loss events per network branch. The entire network requires 25 gain/loss events at 24 sites. We infer that one *Eco*RI site has been lost in parallel in the Hopland and Savannah River genotypes.

These data allow us to address the question of interspecies hybridization and gene flow. As shown in Table 2 and Fig. 2, two mitochondrial genotypes are found among white-tailed deer at the west Texas Longfellow Ranch, Longfellow "A" and "B". These genotypes differ by two events at two sites. Sympatric mule deer at this ranch are genetically indistinguishable from the more common of the two white-tailed deer genotypes (Longfellow "A") at all mapped restriction sites. Also, a survey of another 110 fragments from four endonucleases with tetranucleotide recognition sites (*Hae*III, *Hin*fI, *Hha* I, and *Hpa* II) shows the white-tailed deer and mule deer from this ranch to be identical at all these sites as well. As seen by the number of events distinguishing the genotypes, this common pattern is much more similar to that of typical white-tailed deer from South Carolina than to typical black-tailed deer from California. Allopatric mule deer from the Kimball Ranch, about 75 miles west of the Longfellow Ranch, differ from this common pattern by only one mapped event (Fig. 2).

The closer genetic similarity of west Texas deer to white-tailed deer can also be seen by comparing the sequence divergences among the various genotypes. As shown in Table 3, west Texas deer differ from South Carolina white-tailed deer by only 1.3%, whereas they differ from California black-tailed deer by 5.5%. The extreme divergence observed is 6.9%, between California black-tailed deer and South Carolina white-tailed deer. This is only slightly larger than the California/west Texas divergence. The mtDNA distance data can also be used to estimate the time of separation between these species. If we assume an evolutionary diver-

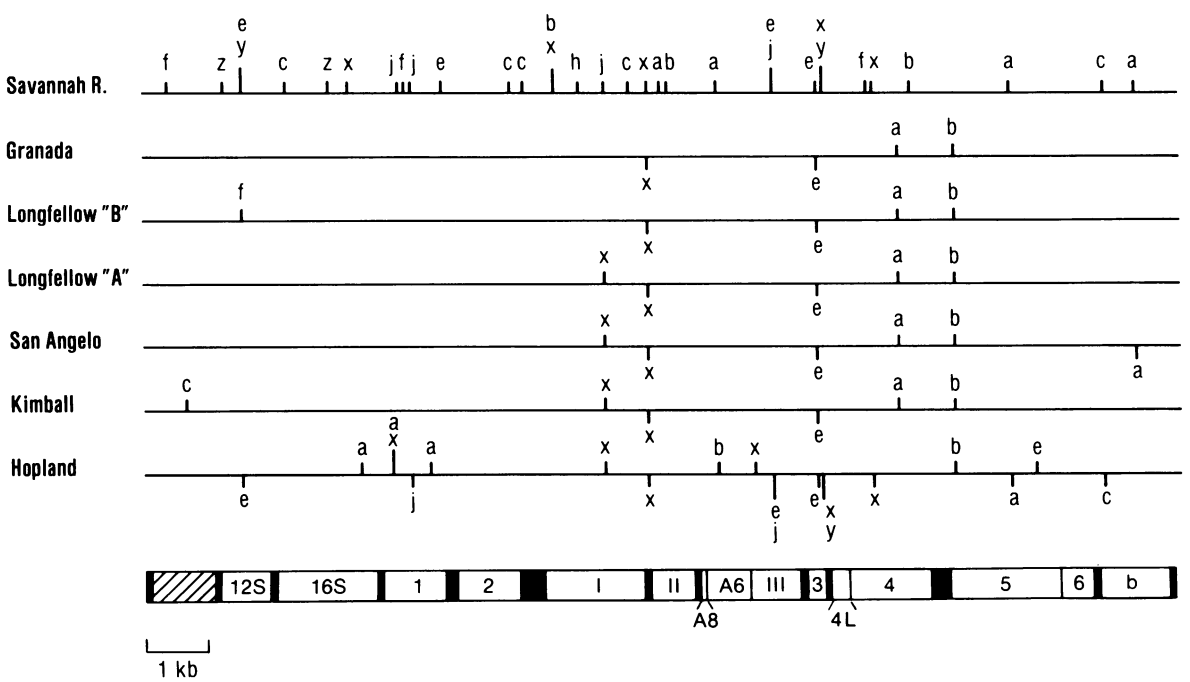

FIG. 1. Restriction maps of seven mtDNA genotypes from deer. The seven genotypes are named after the localities at which they were first found. The uppermost map shows the positions of all sites in the Savannah River white-tailed deer genotype. The height of each vertical line indicates the number of sites mapping to within the same 100-base-pair map unit. The lower maps show the positions of variable sites in the other genotypes with respect to the Savannah River genotype. Those sites present in these genotypes and absent in the Savannah River genotype, and those absent in these genotypes and present in the Savannah River genotype, are indicated by vertical lines above and below the maps, respectively. Restriction endonuclease symbols: a, *Eco*RI; b, *Hin*dIII; c, *Hpa* II; e, *Xba* I; f, *Bam*HI; h, *Pvu* II; j, *Sst* I; x, *Bcl* I; y, *Cla* I; z, *Sst* II. The maps were oriented with respect to the bovine mtDNA sequence (14) at three shared sites; the bar at the bottom shows this mtDNA gene order, for reference. Black areas indicate tRNA genes, the cross-hatched area is a noncoding region including the D-loop, and the numbered and lettered areas indicate genes of known function. kb, Kilobase.

Table 2. Distributions of seven mtDNA genotypes from deer

Species and locality	*n*	No. of deer with mtDNA genotype						
		Sav	Gra	LoB	LoA	San	Kim	Hop
White-tailed deer								
Savannah River Plant, SC	21	21						
Granada Ranch Robertson Co., TX	8		7		1			
San Angelo, Tom Green Co., TX	6					6		
Kerr Co., TX	2		1		1			
Longfellow Ranch, Pecos Co., TX	19			7	12			
Gillespie, Jeff Davis, Jim Wells, Kimble, McLennan, and Uvalde Cos., TX	12				12			
Black-tailed and mule deer								
Longfellow Ranch, Pecos Co., TX	44				44			
Kimball Ranch, Brewster Co., TX	7				1		6	
Hopland Field Station, Mendocino Co., CA	18							18

This table gives, for each locality listed, the number of deer with each of the seven genotypes described in Fig. 1. These genotypes are abbreviated as follows: Sav, Savannah River; Gra, Granada; LoB, Longfellow "B"; LoA, Longfellow "A"; San, San Angelo; Kim, Kimball; Hop, Hopland.

gence rate of 2% per million years per pair of lineages (27), these data agree with albumin immunological distance data (28) in placing this separation at 3–4 million years ago.

DISCUSSION

The sharing of a common mtDNA genotype between sympatric white-tailed and mule deer is strong evidence for interspecies cytoplasmic gene flow. Based on the assumptions that (*i*) mtDNA is maternally inherited and (*ii*) the common pattern is of the white-tailed deer type, we offer the following hypothesis. Gene flow between species in west Texas has occurred by hybridization between white-tailed does and mule deer bucks, with preferential absorption of hybrid offspring into the mule deer gene pool. In such crosses the F_1 hybrid offspring will have equal proportions of white-tailed and mule deer nuclear alleles but will have mtDNA entirely of the maternal, white-tailed deer type. If the F_1 daughters mate with mule deer bucks, the B_1 backcross offspring will have a reduced proportion of white-tailed deer

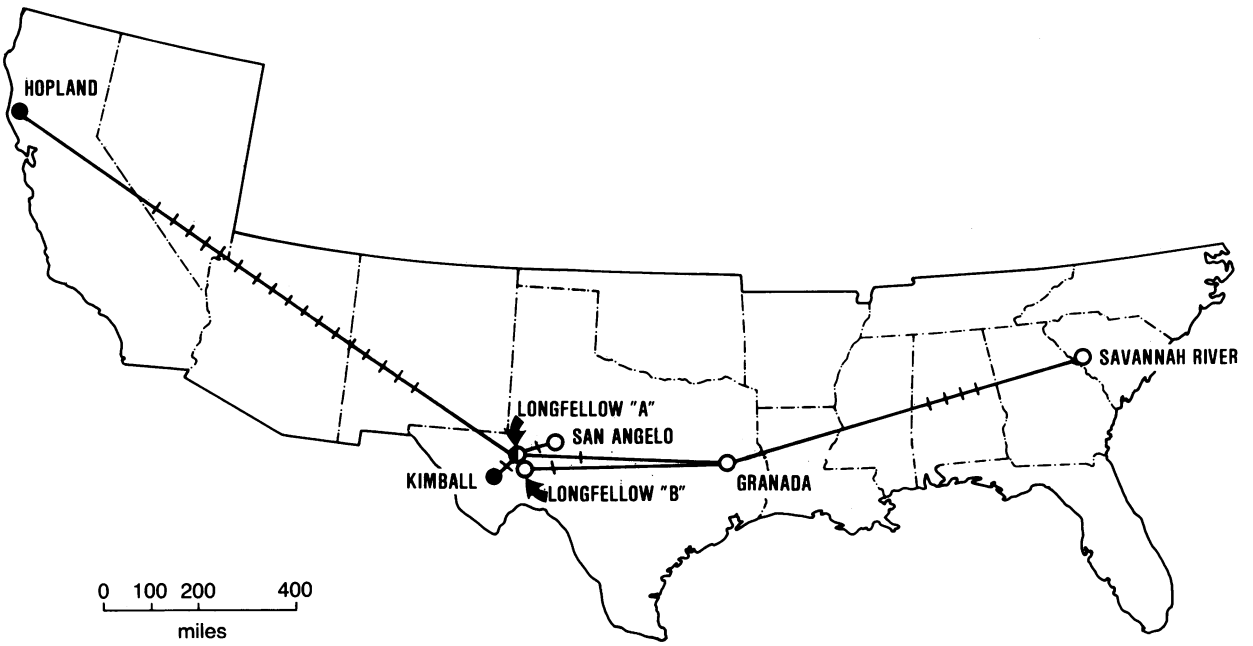

FIG. 2. Geographic and phylogenetic relationships of seven mtDNA genotypes from deer. Genotypes (shown as circles) are designated by the names of the localities at which they were first discovered. Mule deer and black-tailed deer genotypes are indicated by filled circles, and white-tailed deer genotypes, by open circles. The genotypes from the sympatric populations at Longfellow Ranch are indicated by a half-filled circle. The most parsimonious network joining the genotypes is indicated by line segments. The inferred numbers of restriction site gains/losses are indicated by cross-bars on the appropriate line segments. (One mile = 1.6 km.)

Table 3. Sequence divergences among seven mtDNA genotypes from deer

	Sav	Gra	LoB	LoA	San	Kim	Hop
Sav	33	4	5	5	6	6	20
Gra	1.1	33	1	1	2	2	18
LoB	1.3	0.3	34	2	3	3	19
LoA	1.3	0.3	0.5	34	1	1	17
San	1.6	0.5	0.8	0.3	33	2	18
Kim	1.6	0.5	0.7	0.2	0.5	35	18
Hop	6.9	6.0	6.3	5.5	6.0	5.8	31

This table gives the number of sites examined in each genotype (diagonal, underlined); the number of site differences for each pairwise comparison of genotypes (upper half of matrix); and the estimated percent nucleotide sequence divergence (lower half of matrix), based on equation 16 of Nei and Li (25). mtDNA genotypes are abbreviated as in Table 2.

nuclear alleles but will still retain the white-tailed deer mtDNA genotype. Absorption of these offspring into the mule deer population will therefore result in an effective movement of white-tailed deer cytoplasmic genes into the mule deer population against a background of predominantly mule deer nuclear alleles. Once established in the mule deer population, the white-tailed deer mtDNA genotype would be perpetuated as long as the maternal lineage persists.

The social, behavioral, and ecological factors that would establish and maintain hybridization between species are as yet unexplored experimentally. One important consideration is the nature of sex-specific dispersal patterns as they relate to ecology in the areas of sympatry. The social system of white-tailed deer is based on female family groups, each of which comprises an adult doe and her young of from one to several seasons. Typically, bucks disperse from these groups as yearlings (12–24 months) and thereafter establish solitary, permanent residence on new ranges at some distance from those of their mothers. Does, in contrast, tend not to disperse as early but rather are excluded from the maternal group after 24 months. They then establish new home ranges adjacent to or overlapping those of their mothers (29–31). The social system of mule deer and black-tailed deer is basically similar (32, 33). Dispersal patterns and home-range distributions are also influenced by factors such as local productivity and population density. Exact patterns are highly variable geographically within both species (30, 31, 34). Specific data for west Texas deer are unavailable. Studies of niche relationships of deer in the Southwest generally suggest that mule deer and white-tailed deer have similar diets but occupy different habitat types. The former are found typically in xeric, open areas, while the latter usually occur in more mesic areas with denser vegetation (35). In Arizona, encroachment of desert vegetational types into grassland communities, in conjunction with an increasingly hotter and drier climate, has been hypothesized to account for a substantial expansion of desert mule deer into white-tailed deer ranges (36). Mule deer are also characterized as "behaviorally dominant" to white-tailed deer in these areas of sympatry, in part because of their larger size (36). In west Texas, an opposite ecological trend, created by natural expansion of woody brush species in combination with modified patterns of cattle grazing, has increased the density of vegetational cover suitable for white-tailed deer. In consequence, this species has expanded its range at the expense of mule deer (37).

One model supported by the foregoing discussion is that, in west Texas, the expansion of white-tailed deer into newly available habitat has been mediated by short-range dispersal of young does that were excluded from their maternal groups on adjacent ranges. Dispersal by white-tailed yearling bucks, even if it occurred at a relatively higher frequency or over a greater average distance, would be ineffective at moving maternally inherited mtDNA molecules into the mule deer population. Cytoplasmic gene flow in the reverse direction might also be hindered by the failure of dispersing white-tailed bucks either to establish new home ranges in areas already occupied by mule deer bucks or to gain access to mule deer doe mates because of the behavioral dominance of those same bucks. In contrast, dispersing white-tailed does would encounter resident mule deer bucks as mates; the genetic consequences of such meetings would be as described in the above model.

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