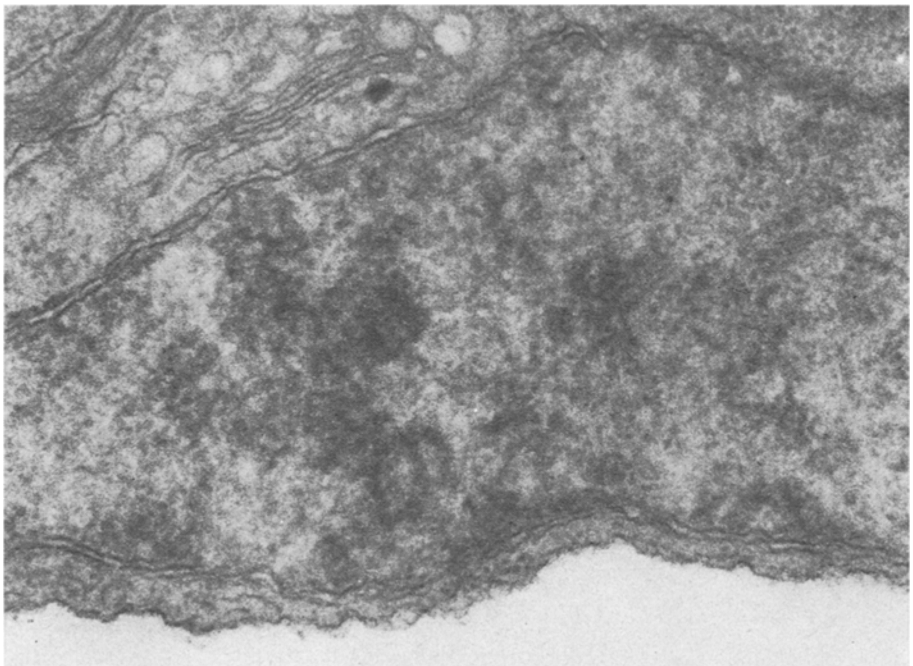

Ultrathin section of a hypocotyl cell nucleus of *Helianthus annuus*. Note the subunit structure throughout the chromatin. Glutaraldehyde, OsO_4, uranylacetate, Epon. ×60,000.

Discussion. The demonstration of chromatin particles in untreated nuclei of the sunflower gives direct evidence for the subunit organization of chromatin, which was so far postulated from results obtained by indirect methods and shown in electron micrographs of specifically pretreated cells. The diameter of the subunit was found to be about 110 Å in *Helianthus*. The statements on the size of nucleosomes given in the literature are variable, such as 69 Å[10], 70–90 Å[12], 125 Å[6], and 135×50 Å[11]. Since it is well established that the single subunit is composed of each 2 molecules of the histones of type f2a1, f2a2, f2b, and f3[2–4], the different diameters reported may be rather the consequence of different techniques employed for their demonstration than the expression of species-specific variation.

Hybridization in the Mexican and 13-Lined Ground Squirrels, *Spermophilus mexicanus* and *Spermophilus tridecemlineatus*

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Summary. Evidence of hybridization between the ground squirrels, *Spermophilus tridecemlineatus* and *S. mexicanus* is presented on the basis of chromosomal and protein data. The hybrids produced appear to be completely interfertile. Based on the evidence of hybridization and the recent reestablishment of contact between these two species, they are considered to be semispecies.

The nearctic ground squirrels of the genus *Spermophilus* are comprised of 6 subgenera or groups of species of close taxonomic affinity[2]. The subgenus *Ictidomys* includes 4 species of striped or spotted ground squirrels, and 2 species *S. mexicanus* and *S. tridecemlineatus* are widespread, occurring in grassy habitats from central Canada and the United States to the eastern Rocky Mountains (*S. tridecemlineatus*) and southwestern United States and northern Mexico (*S. mexicanus*)[3,4]. Recently, NADLER and HUGHES[5] correlated chromosomal characters of the two species, and determined that karyotypic evidence substantiates the close phylogenetic relationship between the two species.

This report provides evidence of natural hybridization between *S. mexicanus* and *S. tridecemlineatus* based on karyological and allozymic variation. Additionally interpretation of the available data provides a basis for determining the level of speciation attained by these two taxa.

Materials and methods. Ground squirrels ($N = 160$) were examined from 18 natural populations as follows: *Spermophilus tridecemlineatus*. Indiana: Terre Haute, Vigo Co., 12; Texas: Denton, Denton Co., 18; Lewisville, Denton Co., 7; Wichita Falls, Wichita Co., 17; Paris,

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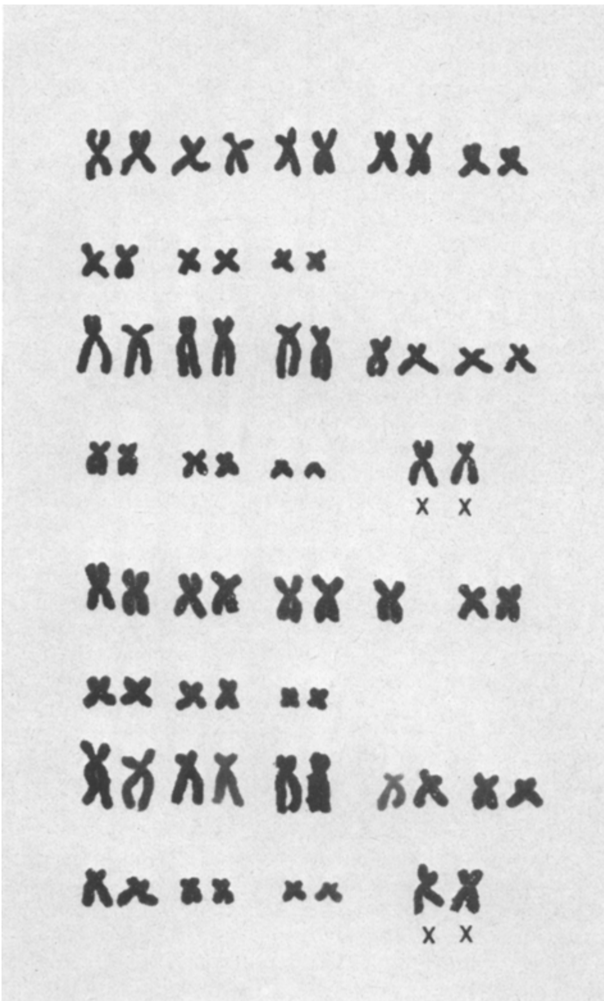

Fig. 1. Karyotypes of *Spermophilus tridecemlineatus*. Note morphology of smallest submetacentric autosomes.

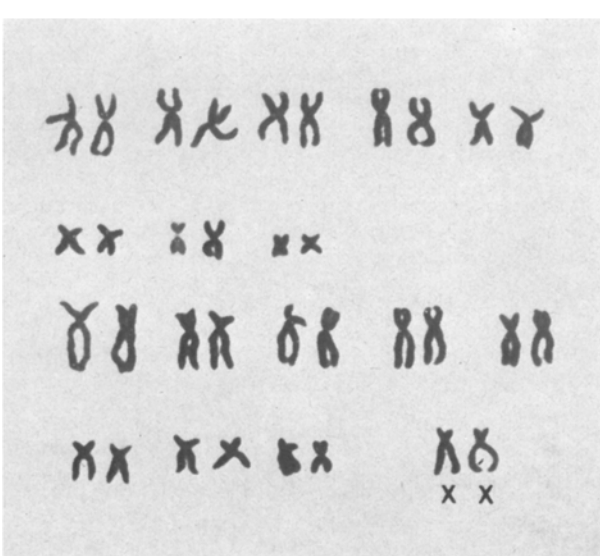

Fig. 2. Karyotype of *Spermophilus mexicanus*

Lamar Co., 10; Corsicana, Navarro Co., 5; Gruver, Hansford Co., 6; Lubbock, Lubbock Co., 5; Amarillo, Potter Co., 5. *Spermophilus mexicanus.* New Mexico: Carlsbad, Eddy Co., 7. Texas: Sheffiled, Pecos Co., 15; Austin, Travis Co., 7; New Braunfels, Comal Co., 5; Midland, Midland Co., 10; Pecos, Reeves Co., 5; Harlengen, Carmeron Co., 7; Corpus Christi, Nueces Co., 9. In addition, 10 specimens were examined from a population of hybrids from Hobbs, Lea Co., New Mexico.

Chromosomes were prepared from bone marrow and testicular material according to the techniques of LEE[6] and ZIMMERMAN[7], respectively. 25 proteins encoded by 28 structural loci were examined utilizing the techniques of RASMUSSEN[8] and Selander et al.[9]. Alleles were scored according to their mobilities, *a* being the fastest, *b* the next fastest, etc. I, the identity measure of NEI[10], was utilized as a measure of identity of genomes between the two species.

Results. 2 chromosomal patterns were found in *S. tridecemlineatus*. The first pattern (A) was similar to that reported for this species in populations from the central United States by NADLER and HUGHES[5], and was the common pattern in ground squirrels from Indiana, New Mexico, and western Texas in this study (Figure 1). The second pattern (B) was observed in populations of *S. tridecemlineatus* from central and southern Texas and differed from the former in that the small pair of acrocentric autosomes is replaced by a small pair of submetacentrics (Figure 1).

The single pattern observed in *S. mexicanus* was identical to that described by NADLER and HUGHES[5]. The major chromosomal difference observed between *S. mexicanus* and *S. tridecemlineatus* is in the size and centromeric position of the 2 smallest pairs of submetacentric autosomes (Figure 2). These pairs are relatively longer, and the centromeres are more medial in *S. mexicanus*.

Electrophoretic results for serum albumins exhibited 3 alleles segregating from a single locus (Figure 3). The Alb^a allele was the most common allele observed in populations of *S. tridecemlineatus* and was fixed in 6 of the 9 populations examined. 3 alleles were also found in *S. mexicanus*. The most common alleles were the Alb^b and Alb^d alleles, although the Alb^c allele also occurred at relatively high frequencies.

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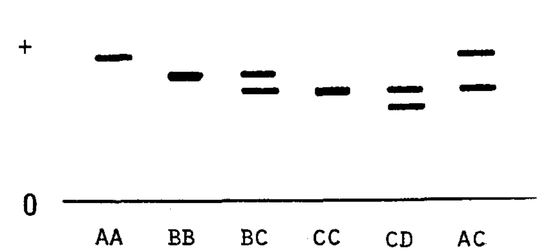

Fig. 3. Electrophoretic patterns of albumin variation in *Spermophilus tridecemlineatus* (slots 1–2) *S. mexicanus* (slots 2–5), and F_1 hybrids between the two species (slot 6).

Fig. 4. Karyotype of F_1 hybrid from *S. tridecemlineatus* × *S. mexicanus* with heteromorphic chromosomes indicated.

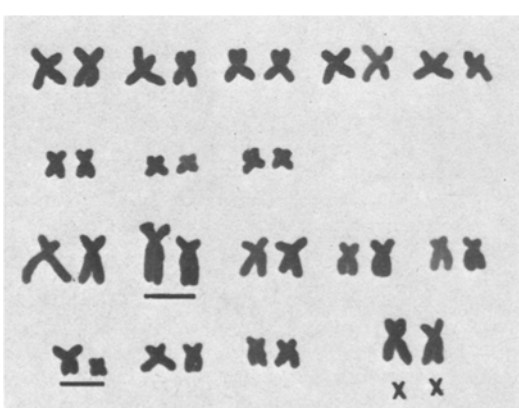

Fig. 5. Karyotype of backcross from F_1 × *Spermophilus mexicanus* with heteromorphic chromosomes indicated (see Figure 4).

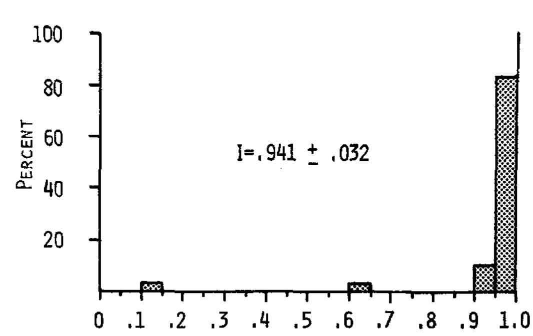

Fig. 6. Frequency distribution of loci relative to genetic identity between *Spermophilus tridecemlineatus* and *S. mexicanus*. I is the mean genetic similarity with its standard error.

The single population from Hobbs, Lea Co., New Mexico produced individuals which were intermediate both in karyotype and albumin patterns. Of the 10 individuals sampled, 4 appear to be hybrids. The hybrid karyotypes are representative of 3 F_1 progeny from a *S. mexicanus* × *S. tridecemlineatus* (karyotype A) cross (Figure 4) and one *S. mexicanus* × F_1 backcross (Figure 5). The karyotypes also demonstrate that there are 3 pairs of chromosomes which differ between the two species. Identification of individual chromosomes of the 3 heteromorphic pairs in Figure 5 indicates the larger chromosomes are from *S. mexicanus*, and the smaller chromosomes are typical of the *S. tridecemlineatus* karyotype.

The 3 individuals with F_1 karyotypes were heterozygous for the Alb^a and Alb^c alleles, the Alb^a allele being the common allele in *S. tridecemlineatus*, and the Alb^c allele being the common allele in *S. mexicanus*. The single individual with a backcross karyotype was heterozygous for the Alb^b and Alb^d alleles.

Discussion. The data presented appear to constitute proof that limited gene exchange is occurring between *S. mexicanus* and *S. tridecemlineatus* in the area of contact of their ranges. More importantly, the hybrids appear to be interfertile. Firstly, one F_1 female was gravid, containing 5 embryos, and 1 hybrid represented a backcross. Secondly, analysis of testicular material from hybrid males revealed numerous sperm and 17 normal bivalents at diakinesis. It is evident that reproductive isolation typical of most species is not complete in these two taxa.

Factors contributing to the reproductive isolation of the species in portions of the range where they come in contact are not readily apparent. Both species frequent areas disturbed by man such as parks, roadsides, and golf courses. Indeed, the area in the vicinity of Hobbs has been so disturbed, and it would appear that hybridization between *S. mexicanus* and *S. tridecemlineatus* is a manifestation of man's production of a 'hybrid habitat' where both species can coexist.

The limited overlap in the ranges of the 2 species, with hybridization in a limited zone of contact leads one to the conclusion that *S. mexicanus* and *S. tridecemlineatus* fit the classical definition of semispecies. According to MAYR[11], semispecies are populations in the second stage of speciation, having differentiated as geographical isolates. Upon establishing secondary contact, semispecies hybridize to a limited extent but retain their distinctiveness as isolated gene pools. Both of these criteria are met by *S. mexicanus* and *S. tridecemlineatus*.

Evidence has accumulated that little differentiation takes place during the second stage of speciation, i.e., the formation of semispecies. AYALA et al.[12] examined genic divergence in 6 semispecies of the *Drosophila paulistorum* group and suggested that the development of reproductive isolation may occur without a concomitant change in a large portion of the genome. The distribution of I values for 29 loci in *S. mexicanus* and *S. tridecemlineatus* are shown in Figure 6, with a mean I of 0.94, and 83% of the loci examined had I values between 0.95 and 1.0. It is clear that relatively little genomic modification has occurred during speciation in these two taxa.

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