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HYBRID ZONES, GENETIC ISOLATION, AND SYSTEMATICS OF POCKET GOPHERS (GENUS *GEOMYS*) IN NEBRASKA

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Pocket gophers of the genus *Geomys* are common inhabitants of many habitats throughout most of the state of Nebraska. Because the taxonomic history of *Geomys* has undergone numerous changes through the years, these pocket gophers have been the subjects of ongoing taxonomic and distributional studies and in more recent years genetic studies to understand relationships among populations. In order to gain deeper insight into the relationships among these taxa of *Geomys*, we intensively collected specimens from areas where chromosomal races were thought to form contact zones. Results from examination of genetic (chromosomes, mitochondrial cytochrome-*b* gene sequences, and nuclear interphotoreceptor retinoid-binding protein gene sequences), morphometric, and pelage coloration data revealed 2 areas of hybridization between taxa of *Geomys* in Nebraska. The 1st of these corresponded to the Oakdale vicinity in Antelope and Madison counties in northeastern Nebraska and the 2nd corresponded to Lincoln County in southwestern Nebraska. The taxonomic implications of our study support the recommendations from earlier studies performed in other areas of the geographic range of *Geomys*. Specifically, in Nebraska we recognize 3 species: *G. bursarius majusculus* in eastern Nebraska, *G. lutescens* in the Sand Hills and adjacent areas of central and western Nebraska, and *G. jugossicularis halli* in southwestern Nebraska. The exact geographic distributions and relationships of these species within Nebraska and the surrounding states remain to be determined in detail.

Key words: chromosomes, genetic isolation, *Geomys*, hybrid zone, interphotoreceptor retinoid-binding protein gene, mitochondrial cytochrome-*b* gene, Nebraska, systematics

Pocket gophers are widespread and common inhabitants of a variety of habitats throughout most of the state of Nebraska. They occur from the broad river valleys along the Platte, Elkhorn, Missouri, Niobrara, and Republican rivers, throughout the Sand Hills of the west-central part of the state, to the pine-clad hills of the Pine Ridge and Wildcat Hills of the far western parts of the state. These pocket gophers have been the subjects of a number of taxonomic and distributional studies and in more recent years genetic studies to understand relationships among populations. Hayden (1875:93) was 1st to report pocket gophers from Nebraska, using the name *Geomys bursarius*, when he found them to be ''very abundant on the rich bottoms around Council Bluff [Washington County]'' and took a specimen ''on the Niobrara'' River, probably in current Knox County.

Merriam (1890) was the 1st to apply a new name to populations of *Geomys* in Nebraska when he described *G. bursarius lutescens* based on a specimen collected by A. B. Baker along Birdwood Creek in the Sand Hills of Lincoln County (probably near 10.5 km north and 2.5 km east of Sutherland). He distinguished the new taxon from *G. bursarius* of the Mississippi Valley by its paler coloration and smaller size. In a monographic revision of the family, Merriam (1895) treated *G. bursarius* and *G. lutescens* as distinct species. Merriam assigned specimens from 6 localities in eastern and northern Nebraska to *G. bursarius* and specimens from 16 localities in central and western Nebraska (primarily associated with the Sand Hills region), as well as specimens from Wyoming, Kansas, Oklahoma, and Texas to *G. lutescens*. Over the next 50 years, authors vacillated between treating these

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2 taxa as distinct species or subspecies of a single species, although a majority favored the former arrangement (Swenk 1908, 1918, 1939, 1940). However, following the work of Villa-R. and Hall (1947) on pocket gophers in Kansas, *G. lutescens* was treated as a subspecies of the older named taxon *G. bursarius*. Villa-R. and Hall (1947:219–220) stated: ''intergradation has been found to occur between every pair of kinds having contiguous geographic ranges. The characters previously thought by some writers constantly to differentiate, say *Geomys lutescens* of western Kansas from *Geomys bursarius* of eastern Kansas, prove not to do so.''

In the late 1930s and early 1940s, 4 additional populations of pocket gophers in Nebraska were given subspecific recognition. Blossom (1938) described *G. l. hylaeus* based on specimens from the Pine Ridge south of Chadron, Dawes County, in northwestern Nebraska, distinguishing this new taxon based on its smaller size and darker coloration (especially middorsally), than in typical *G. lutescens*. Swenk (1939, 1940) studied size variation in *Geomys* in Nebraska and described 1 new subspecies of *G. bursarius* and 2 of *G. lutescens*. Swenk (1939) described *G. b. majusculus* from a type locality at Lincoln, Lancaster County, and estimated its geographic range as eastern Nebraska, western and southern Iowa, northeastern Kansas, and southeastern South Dakota. He distinguished this subspecies based on the larger size of old adults compared with typical *G. b. bursarius* of Minnesota, Wisconsin, and Missouri. Swenk (1940), in an intensive morphological study of *G. lutescens* in Nebraska, recognized 2 new subspecies with geographic ranges at the periphery of the Sand Hills. He described *G. l. levisagittalis* from Spencer, Boyd County, in the drainage of the Niobrara River in northeastern Nebraska. Although Swenk only had specimens from the type locality, he believed that this form probably occurred elsewhere in Boyd County and adjacent South Dakota in sandy soils and stream bottoms. He distinguished *levisagittalis* by its larger body size and details of its cranium, including flattening of the braincase and a broader and heavier rostrum. The 2nd subspecies of *G. lutescens* described by Swenk (1940) was *vinaceus*, which was distinguished primarily based on a smaller skull and color of the upper parts of the body. This subspecies had a type locality at Scottsbluff, Scotts Bluff County, in extreme western Nebraska and was found to occur in the sandy valleys of the High Plains to the west and south of the Sand Hills.

Almost as soon as these new taxa were described, other researchers were moving them into synonymies of older names. Russell and Jones (1956) placed *G. l. vinaceus* as a junior synonym of *G. b. lutescens*, concluding that no significant difference in size existed between the 2 and that *vinaceus* represented only minor variations within a local population. Jones (1964) placed both *G. l. hylaeus* and *G. l. levisagittalis* as junior synonyms of *G. b. lutescens* and considered the specimens from the type locality of *levisagittalis* to represent intergrades between *G. b. lutescens* and *G. b. majusculus* and thus not worthy of taxonomic recognition. Jones (1964) attributed the diagnostic characters that formed the basis for the recognition of *G. l. hylaeus* to an age bias in the individuals studied by Blossom (1938), with the resulting expected variation with age not being worthy of taxonomic recognition. Therefore, Jones (1964) recognized a single species of *Geomys* as occurring in Nebraska—*G. bursarius* with only 2 subspecies, *majusculus* in the eastern one-third of the state, and *lutescens* in the western two-thirds.

A series of 4 papers in the early 1980s (Heaney and Timm 1983, 1985; Timm et al. 1982; Timm and Price 1980) brought together data from morphometrics, karyology, ectoparasites, and anatomy of the glans penis and baculum to analyze the relationships of *Geomys* on the central and northern Great Plains. These investigators recognized 2 species of *Geomys* in Nebraska, *G. lutescens* in western parts of the state and *G. bursarius* in the east. Heaney and Timm (1983:55; 1985) found ''Hybridization between *G. bursarius* and *G. lutescens* occurs only at a single locality in Nebraska and that introgression there is inconsequential.'' This site is in the vicinity of Oakdale in Antelope County in northeastern Nebraska. Within *G. bursarius*, 3 subspecies were recognized, with *G. b. bursarius* as a ''widespread, variable'' subspecies (Heaney and Timm 1983:55) occurring in Nebraska. This action placed *G. b. majusculus* as a junior synonym of the nominate subspecies. The geographic range of *G. lutescens* was shown to extend as far south as Oklahoma, with the nominate subspecies occurring in Nebraska and northwestern Kansas and *G. l. major* occurring in southwestern Kansas and Oklahoma.

Burns et al. (1985) used morphometric, bacular, karyotypic, and electrophoretic analyses to examine the same contact zone in Nebraska and another potential zone in Kansas between eastern and western populations of *Geomys*. They concluded that introgression was occurring at the Nebraska site and, therefore, a single species (*G. bursarius*) should be recognized in Nebraska and Kansas. Sudman et al. (1987), recognizing only *G. bursarius*, examined the relationships of these pocket gophers in southwestern Nebraska and adjacent parts of Kansas. They found karyotypic, morphometric, and color differences between populations of *G. b. lutescens* from north and south of the Platte River and applied the new taxonomic name *G. b. halli* to populations south of the Platte River. The ranges of these 2 subspecies approach each other along the valleys of the North Platte and South Platte rivers before their confluence just east of the town of North Platte along the Platte River in Lincoln County.

The most recent work on this group of pocket gophers (Sudman et al. 2006) examined sequences of the mitochondrial cytochrome-*b* gene (*Cytb*). Although their study did not directly consider relationships among populations of *Geomys* in Nebraska, they did assign specimens from Nebraska (Fig. 1) to 3 species—*G. bursarius majusculus*, *G. lutescens*, and *G. jugossicularis halli*. The first 2 taxa are those that have been extensively studied in the past; however, the taxon *jugossicularis* initially was described as a subspecies of *G. lutescens* (Hooper 1940) and subsequently treated as a subspecies of *G. bursarius*. This arrangement placed the taxon *major* again as a subspecies of *G. bursarius* and would appear to apply *G. jugossicularis* to populations in southwestern Nebraska, western Kansas, eastern Colorado (type locality at Lamar, Prowers County), and the Oklahoma Panhandle.

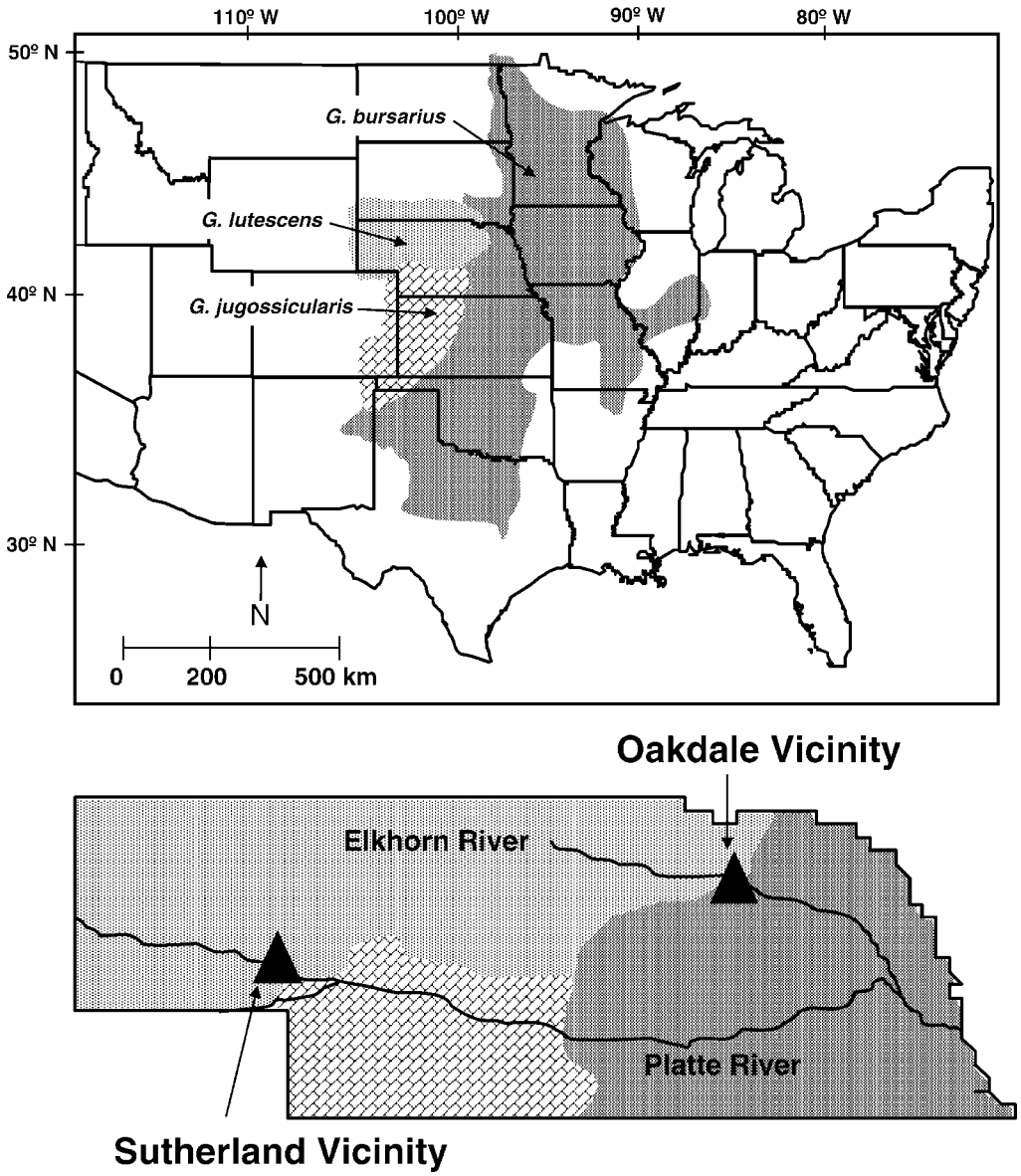

Fig. 1.—Map depicting the distribution of the 3 species of pocket gophers (*Geomys*) examined in this study. An inset of Nebraska (not to scale) is provided to depict the collecting localities referenced in the text.

In order to gain deeper insight into the relationships among these taxa of *Geomys*, we intensively collected at contact zones in Antelope and Lincoln counties in Nebraska. Specimens were collected for a range of studies including morphometrics, karyology, and molecular analyses of the mitochondrial and nuclear genomes.

Materials and Methods

Sampling.—Seventy-six pocket gophers were livetrapped at 2 separate hybrid zones in Nebraska using Baker–Williams gopher traps (Baker and Williams 1972). The 1st zone is in Antelope and Madison counties near the communities of Neligh, Oakdale, Meadow Grove, and Tilden (Fig. 2). The 2nd zone is located in Lincoln County near the communities of Sutherland and Maxwell (Fig. 3). These 2 general areas sit at the margin of the Sand Hills region, an area of approximately 19,300 square miles of wind-blown sand dunes in central and western Nebraska. This is the largest sand dune area in the Western Hemisphere (Bleed and Flowerday 1990), with soils dominated by eolian sands with poorly developed topsoil and little or no subsoil (Lewis 1990; Swinehart 1990). The Sand Hills are covered with a mixed-grass prairie in which the vegetation usually is not so dense that the ground cannot be seen. In this region, trees are confined to the major watercourses (Kaul et al. 2006). Populations of *G. lutescens* occupy most of the Sand Hills region and some of the surrounding areas. Heaney and Timm (1983) identified a site about 1.5 km west of Oakdale, Antelope County, at which they found evidence that *G. lutescens* and *G. bursarius* were hybridizing. This locality is one of the easternmost extensions of the Sand Hills. The pale eolian sandy soils of the Sand Hills encounter

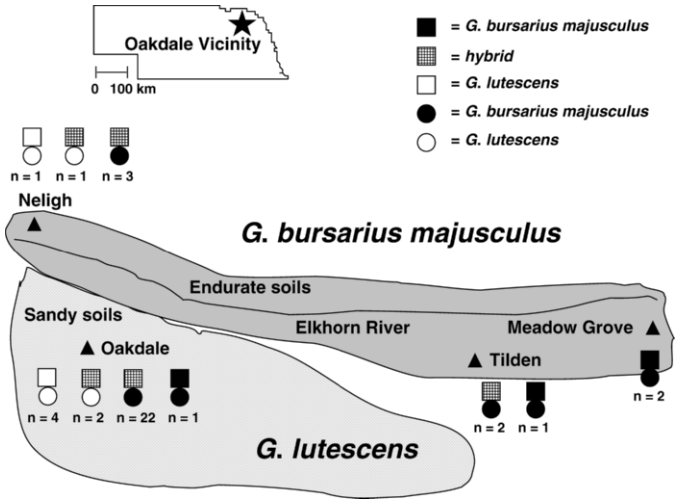

Fig. 2.—Map depicting the hybrid zone (not to scale) referred to in the text as the Oakdale locality. Shaded areas illustrate soil types. An ''n'' refers to the number of individuals possessing a particular genotype. A star indicates the general location of the hybrid zone; triangles indicate towns (collecting localities identified in the text), and squares depict nuclear genotypes (chromosome and *Rpb*3 data) and circles refer to mitochondrial haplotypes.

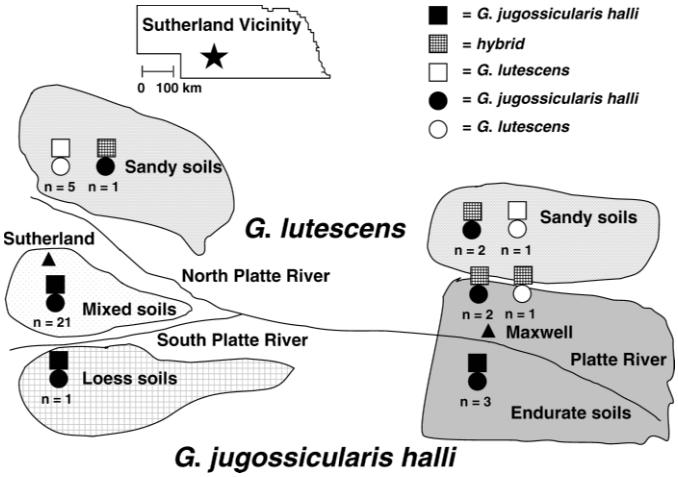

Fig. 3.—Map depicting the hybrid zones (not to scale) referred to in the text as the Sutherland and Maxwell localities. Shaded areas illustrate soil types. An ''n'' refers to the number of individuals possessing a particular genotype. A star indicates the general location of the hybrid zone; triangles indicate towns (collecting localities identified in the text), and squares depict nuclear genotypes (chromosome and *Rpb*3 data) and circles refer to mitochondrial haplotypes.

and overlay the dark, silty loam soils that occur in the Elkhorn River Valley. We conducted survey work in an east–west direction through this point beginning near Meadow Grove in Madison County and terminating south of Neligh in Antelope County, about 8 km west of Oakdale. Populations of *G. bursarius* generally occurred in the dark, silty loam soils to the east and southeast of this area.

Sudman et al. (1987), studying karyological differences among populations of *Geomys* on the central Great Plains, found that 2 chromosomal races met along the Platte River complex in Lincoln County, Nebraska. They described the taxon *halli* from south of the river, but placed it in the single species (*G. bursarius*) that they recognized throughout the state; however, intergradation was never demonstrated between populations lying north and south of the river. East of Lincoln County the chromosomal races were separated by a 40-km gap formed by the heavy loess soils north of the Platte River. However, in Lincoln County this distributional gap is missing because the Sand Hills meet the north shore of the river. Trapping for pocket gophers was conducted in 2 north–south regions in Lincoln County. One of these passed through the town of Sutherland in the western part of the county and the other through Maxwell in the eastern part.

The North Platte and South Platte rivers merge just east of the town of North Platte to form the Platte River. The town of Sutherland lies between the North Platte and South Platte rivers before their confluence at a point where the Sand Hills approach within 100 m or less of the North Platte River. Trapping began within the eolian sandy soils north of the river and crossed the North Platte River proceeding through Sutherland across the South Platte River and into the loess hills that border the river valley. Sutherland is positioned on the rise that separates the rivers, with soils between the rivers being dominated by a mixture of coarse river sand and gravel with silt eroded from upstream. Trapping was conducted wherever pocket gophers were observed through this area. A significant number of specimens were taken along the north side of the North Platte irrigation canal, where excavated soil from the canal was placed to create a maintenance road. The town of Maxwell is between the north side of the Platte River and the edge of the Sand Hills, which approach to within 2 km of the river. This transect extended from the Sand Hills to north of Maxwell across the river and onto the broad floodplain south of the river. Floodplain soils were dark, silty loams.

The geographic range of *G. jugossicularis halli* extends south of the Platte River through southwestern Nebraska and into adjacent Colorado and Kansas. Beyond the loess hill along the southern edge of the Platte River valley, another series of sandy dunes is encountered that extends as the Wray Dune Field into northeastern Colorado and nearly reaches the Front Range (Muhs 1985). Recent research indicates that these dunes are similar in age and composition to the Sand Hills north of the river (Muhs 1985). The vegetation in the northern portion of these dunes is a mixed-grass prairie similar to that of the Sand Hills, but progresses into sand sage mixed-grass prairie in extreme southwestern Nebraska, Colorado, and Kansas. This vegetation is similar to that of the sandhills mixed-grass prairie, but contains considerable sand sagebrush (Kaul et al. 2006; Ramaley 1939).

Cytochrome-b data set.—Nucleotide sequence variation from *Cytb* was examined from 2 individuals considered as parental types (not from potential hybrid zone areas) of *G. b. majusculus* (TTU76065, Meadow Grove locality, and TTU76066, Tilden locality) and *G. j. halli* (TTU76069 and TTU76071, Sutherland locality), and 1 individual of *G. lutescens* (TTU76077, Maxwell locality). GenBank accession

Table 1.—Summary of specimens and data sets examined in this study. Information for each specimen includes Texas Tech University unique museum number (TK no.) and museum catalogue number (MCN; TTU = Museum of Texas Tech University and UNSM = University of Nebraska State Museum), collecting locality, sex (f = female and m = male), age (A = adult and J = juvenile), condylobasal length (CBL, in mm), pelage coloration (PC, scored 1–5), mitochondrial DNA haplotype (mtDNA, based on *Mbo*I digest of the cytochrome-*b* gene [*Cytb*]), diploid number (2n), fundamental number (FN), overall chromosome type (OC), interphotoreceptor retinoid-binding protein (*Rbp3*) based on DNA sequences of the interphotoreceptor retinoid-binding protein exon I, nuclear genotype (Nuc) developed from chromosome and interphotoreceptor retinoid-binding protein data, and an overall genotype (OG) based on mtDNA, chromosomes, and interphotoreceptor retinoid-binding protein data. Abbreviations are: M = *G. b. majusculus*, L = *G. lutescens*, H = *G. j. halli*, * = hybrid, BCL = backcross to *G. lutescens*, and BCM = backcross to *G. b. majusculus*. Interpretation of chromosome data is as follows: 2n = 70 and FN = 68, *G. b. majuscules*; 2n = 72 and FN = 86–98, *G. lutescens*; and 2n = 72 and FN = 70, *G. j. halli*.

TK no./MCN	Collecting locality	Sex	Age	CBL	PC	*Cytb*	2n	FN	OC	*Rbp3*	Nuc	OG
52255/UNSM20839	Antelope Co., 7.2 km S Neligh	f	A	43.50	2	L	72	92–94	L	M/L	BCL	*
52258/UNSM20836	Antelope Co., 7.2 km S Neligh	m	A	49.80	2	M	72	93–95	L	M	BCM	*
52261/UNSM20835	Antelope Co., 8.0 km S Neligh	f	J	38.00	3	M	72	88–90	L	L	BCL	*
52263/UNSM20843	Antelope Co., 7.2 km S Neligh	m	J	36.00	1	L	72	88–90	L	L	L	L
52264/UNSM20838	Antelope Co., 7.2 km S Neligh	m	A	49.70	1	M	72	86–88	L	L	BCL	*
52259/UNSM20844	Antelope Co., 1.6 km E Oakdale	m	A	49.40	3	L	72	72	*	L	BCL	*
54369/ TTU76098	Antelope Co., 1.6 km E Oakdale	f	A	44.00	3	M				L	BCL	*
52260/UNSM20842	Antelope Co., 0.8 km W Oakdale	m	A	49.40	3	L	72	71	*	L/M	BCL	*
52256/UNSM20841	Antelope Co., 1.1 km W Oakdale	f	A	41.00	3	M	71	89	*	L	BCL	*
52257/UNSM20837	Antelope Co., 1.1 km W Oakdale	f	A	45.80	3	M	71	81–83	*	M	BCM	*
52262/UNSM20840	Antelope Co., 1.1 km W Oakdale	f	A	41.20	3	M	70	88	*	L	BCL	*
54277/TTU76047	Antelope Co., 1.1 km W Oakdale	f	A	37.70	1	L	72	86–88	L	L	L	L
54283/TTU76044	Antelope Co., 1.1 km W Oakdale	f	A	40.75	3	M	71	88–91	*	M	BCM	*
54284/TTU76048	Antelope Co., 1.1 km W Oakdale	f	A	47.30	2	M	70	86	*	L	BCL	*
54285/TTU76046	Antelope Co., 1.1 km W Oakdale	f	J	36.80	2	M	70	88	*	M	BCM	*
54289/TTU76045	Antelope Co., 1.1 km W Oakdale	m	A	38.95	1	M	70	84–86	*	L	BCL	*
54307/TTU76052	Antelope Co., 1.1 km W Oakdale	f	A	43.45	3	M				L	BCL	*
54323/TTU76053	Antelope Co., 1.1 km W Oakdale	m	J	33.35	2	L	72	86–88	L	L	L	L
54324/TTU76055	Antelope Co., 1.1 km W Oakdale	f	A	44.15	2	L				L	L	L
54326/TTU76049	Antelope Co., 1.1 km W Oakdale	f	A	45.50	3	M	71	80–82	*	M	BCM	*
54332/TTU76054	Antelope Co., 1.1 km W Oakdale	m	A	40.45	2	M	70	86–88	*	L	BCL	*
54335/TTU76050	Antelope Co., 1.1 km W Oakdale	f	A	47.60	2	M	72	90–94	L	L	BCL	*
54336/TTU76060	Antelope Co., 1.1 km W Oakdale	f	A	43.30	2	M	72	90–94	L	M/L	BCL	*
54356/TTU76056	Antelope Co., 1.1 km W Oakdale	f	A	35.60	1	M	71	79–84	*	M	BCM	*
54357/TTU76062	Antelope Co., 1.1 km W Oakdale	m	A	35.95	1	M	70	81–84	*	M	BCM	*
54359/TTU76057	Antelope Co., 1.1 km W Oakdale	f	A	41.75	2	M	72	84–86	*	L	BCL	*
54361/TTU76059	Antelope Co., 1.1 km W Oakdale	m	J	33.60	2	M	71	88–90	*	L	BCL	*
54362/TTU76061	Antelope Co., 1.1 km W Oakdale	f	J	33.00	1	M	72	80–84	*	M/L	BCL	*
54411/TTU76058	Antelope Co., 1.1 km W Oakdale	m	A	36.70	1	L	72	88	L	L	L	L
54412/TTU76063	Antelope Co., 1.1 km W Oakdale	m	A	40.15	1	M	71	88	*	L	BCL	*
54334/TTU76051	Antelope Co., 1.1 km W Oakdale	m	J	36.60	1	M	72	95	L	L	BCL	*
54344/TTU76095	Antelope Co., 4.5 km W Oakdale	f	A	42.70	2	M				L	BCL	*
54368/TTU76096	Antelope Co., 4.8 km W Oakdale	f	A	50.40	2	M				M	M	M
54410/TTU76097	Antelope Co., 4.8 km W Oakdale	m	A	42.60	1	M	70	88	*	L	BCL	*
54297/TTU76067	Madison Co., 2.7 km W Meadow Grove	m	J	40.10	2	M	70	68	M	M	M	M
54333/TTU76068	Madison Co., 2.7 km W Meadow Grove	f	A	54.55	5	M	70	68	M	M	M	M
54342/TTU76065	Madison Co., 1.6 km E Tilden	f	A	49.40	5	M	72	84	L	M/L	BCL	*
54349/TTU76066	Madison Co., 1.6 km E Tilden	m	A	37.35	2	M	70	68	M	L	BCL	*
54367/TTU76064	Madison Co., 1.6 km E Tilden	m	A	58.30	5	M	70	68	M	M	M	M
52494/TTU77961	Lincoln Co., 0.3 km E Sutherland	m	A	42.05	3	H	72	70	H	H	H	H
52247/TTU77952	Lincoln Co., 0.8 km E Sutherland	f	A	43.60	2	H				H	H	H
54464/TTU76069	Lincoln Co., 1.1 km N Sutherland	m	J	35.15	1	H	72	70	H	H	H	H
54477/TTU76070	Lincoln Co., 1.6 km N Sutherland	m	A	44.60	2	H	72	70	H	H	H	H
54465/TTU76071	Lincoln Co., 2.6 km N Sutherland	f	A	41.10	3	H	72	70	H	H	H	H
52340/UNSM20856	Lincoln Co., 2.9 km N Sutherland	f	A	41.00	3	H	72	70	H	H	H	H
52342/UNSM20852	Lincoln Co., 2.9 km N Sutherland	f	A	40.20	4	H	72	70	H	H	H	H
52343/UNSM20853	Lincoln Co., 2.9 km N Sutherland	f	A	42.10	3	H	72	70	H	H	H	H
52345/UNSM20850	Lincoln Co., 2.9 km N Sutherland	f	A	42.70	4	H	72	70	H	H	H	H
52347/UNSM20851	Lincoln Co., 2.9 km N Sutherland	f	J	35.60	4	H	72	70	H	H	H	H
52339/UNSM20855	Lincoln Co., 3.2 km S Sutherland	m	A	48.10	4	H	72	70	H	H	H	H
52496/TTU77962	Lincoln Co., 5.1 km N Sutherland	m	A	45.40	3	H	72	70	H	H	H	H
52244/TTU77954	Lincoln Co., 5.6 km N Sutherland	m	A	44.85	2	H	72	70	H	H	H	H
52500/TTU77958	Lincoln Co., 5.6 km N Sutherland	m	A	44.20	4	H	72	70	H	H	H	H
52246/TTU77956	Lincoln Co., 5.6 km N Sutherland	f	A	38.35	3	H	72	70	H	H	H	H

TABLE 1.—Continued.

TK no./MCN	Collecting locality	Sex	Age	CBL	PC	*Cytb*	2n	FN	OC	*Rbp3*	Nuc	OG
52495/TTU77955	Lincoln Co., 5.6 km N Sutherland	f	A	39.15	3	H	72	70	H	H	H	H
52497/TTU77957	Lincoln Co., 5.6 km N Sutherland	f	A	40.30	4	H	72	70	H	H	H	H
52245/TTU77963	Lincoln Co., 5.9 km N Sutherland	m				L	72	96–98	L	L	L	L
52243/TTU77959	Lincoln Co., 6.1 km N Sutherland	f	A	41.30	2	L	72	96–98	L	L	L	L
52499/TTU77960	Lincoln Co., 6.1 km N Sutherland	f	A	41.35	2	H	72	94–96	L	L/H	BCL	*
54482/TTU76073	Lincoln Co., 2.6 km N, 0.8 km E Sutherland	f	A	42.70	3	H	72	70	H	H	H	H
54483/TTU76072	Lincoln Co., 2.6 km N, 0.8 km E Sutherland	f	A	42.25	4	H	72	70	H	H	H	H
54484/TTU76074	Lincoln Co., 2.6 km N, 0.8 km E Sutherland	f	A	42.25	3	H	72	70	H	H	H	H
52498/TTU77953	Lincoln Co., 4.0 km N, 0.3 km E Sutherland	m	A	41.85	3	H	72	70	H	H	H	H
52242/TTU77951	Lincoln Co., 4.0 km N, 0.8 km E Sutherland	m	A	44.75	3	H	72	70–72	H	H	H	H
52341/UNSM20857	Lincoln Co., 7.2 km N, 4.8 km E Sutherland	f			2	L	72	96–98	L	L	L	L
52344/UNSM20854	Lincoln Co., 7.2 km N, 4.8 km E Sutherland	m	J	35.40	2	L	72	95–98	L	L	L	L
52346/UNSM20858	Lincoln Co., 7.2 km N, 4.8 km E Sutherland	m	A	41.90	1	L	72	86–90	L	L	L	L
54463/TTU76075	Lincoln Co., 0.2 km S, Maxwell (N bank)	f	A	43.95	2	H	72	90–92	*	L	L	*
54466/TTU76076	Lincoln Co., 0.3 km S, Maxwell (N bank)	f	A	46.55	2	H	72	92–94	*	L/H	BCL	*
54481/TTU76077	Lincoln Co., 1.4 km S, Maxwell (N bank)	m	J	35.55	1	L	72	94	*	H	BCH	*
54467/TTU76079	Lincoln Co., 1.6 km S, Maxwell (S bank)	m			3	H				H	H	H
54468/TTU76078	Lincoln Co., 1.6 km S, Maxwell (S bank)	f	J	35.45	1	H				H	H	H
54479/TTU76080	Lincoln Co., 1.6 km S, Maxwell (S bank)	f	A	43.30	2	H	72	70	H	H	H	H
54469/TTU76083	Lincoln Co., 2.4 km N Maxwell	m	A	42.10	2	H				L	L	*
54478/TTU76081	Lincoln Co., 2.4 km N Maxwell	f	A	42.55	2	H	72	87–89	L	L	L	*
54480/TTU76082	Lincoln Co., 2.4 km N Maxwell	m	A	46.45	2	L	72	90–94	L	L	L	L

numbers for these specimens are EU332153–EU332157. Mitochondrial DNA was extracted from approximately 0.1 g of frozen liver tissue and amplified using the polymerase chain reaction method following Saiki et al. (1988). Primers located in the flanking tRNAs (5′-MVZ05—Smith and Patton 1993) and (3′-H15915—Irwin et al. 1991) were used to amplify the complete *Cytb* region (1,143 base pairs [bp]). Polymerase chain reaction was performed for 27 cycles using the following parameters: 95°C denaturation (1 min), 50°C annealing (1 min), and 72°C extension (2 min). Polymerase chain reaction products were ligated and cloned using Bluescript plasmids (Stratagene, La Jolla, California) modified with a T nucleotide overhang. DNA sequences were obtained using the dideoxy chain-termination method (Sanger et al. 1977) and 8 primers, the 2 primers used in the polymerase chain reaction amplification (H15915 and MVZ05) and 6 internal primers (400R, 700L, and WDRAT 1100 [Peppers and Bradley 2000]; and 400F, WDRAT 650, and CWE1 [Edwards et al. 2001]). In consideration of the potential for *Taq* polymerase error, multiple clones were sequenced for all individuals. Sequences were aligned using visual inspection and the MacVector 4.17 program (Oxford Molecular, Oxford, United Kingdom).

Given that *Cytb* sequences have been shown to be taxon-specific in several mammalian studies, sequences corresponding to each parental type were examined for diagnostic restriction fragment patterns using the Cut Site Map option in MacVector 4.17 program (Oxford Molecular). Based on restriction fragment patterns (haplotype), *Mbo*I was used to identify polymorphisms that were taxon-specific: *G. b. majusculus* possessed *Mbo*I sites at *Cytb* positions 844 and 990; *G. lutescens* at positions 100, 313, 610, and 844; and *G. j. halli* at positions 100, 313, 610, 844, and 990. Mitochondrial DNA was isolated from all remaining samples ($n = 71$), the *Cytb* region amplified as above, and polymerase chain reaction products were digested with *Mbo*I following the manufacturer's directions (Promega, Madison, Wisconsin). Digested fragments were separated on 0.8% agarose gels, photographed, and assigned to the appropriate taxon.

Interphotoreceptor retinoid-binding protein (Rbp3) data set.—Genomic DNA was extracted from approximately 0.1 g of frozen liver tissue using a DNeasy Kit (Qiagen, Valencia, California) for 12 parental type individuals (3 *G. b. majusculus*, 4 *G. lutescens*, and 5 *G. j. halli*). Approximately 1,300 bp near the 5′ end of exon 1 of the *Rbp*3 gene was amplified by polymerase chain reaction methods with primers modified from Stanhope et al. (1992). AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, California) was used with the following profile: initial denaturation at 95°C (10 min), 35 cycles at 95°C (25 s), 58°C (20 s), 72°C (1 min), and a final elongation of 72°C (10 min). Resulting amplicons were purified using a Qiagen PCR Purification kit (Qiagen) and used as templates in cycle-sequencing reactions using 6 internal primers (modified from Stanhope et al. [1992]) in addition to the primers used in polymerase chain reaction amplification. Sequences were generated on an ABI 3100-*Avant* Genetic Analyzer (Applied Biosystems), aligned and proofed using Sequencher 4.1 software (Gene Codes, Ann Arbor, Michigan), and were deposited in GenBank (accession numbers EU333406–EU333417).

From these initial sequences, a 450-bp region near the 3′ end of the amplified fragment was found to contain 3 nucleotide sites that were diagnostic for each of the 3 taxa. Consequently, this 450-bp region was sequenced in all 64 individuals from the hybrid zones using the 1405R and E2 primers of Stanhope et al. (1992). Heterozygous sites were coded following the

International Union of Biochemistry polymorphism code. GenBank accession numbers are EU333418–EU333481.

Chromosome data set.—Karyotypes were prepared following the methods of Baker and Qumsiyeh (1988). The diploid number (2n) and the fundamental number (FN) were determined by scoring a minimum of 3 metaphase chromosomal spreads for each individual. Following Heaney and Timm (1983), Timm et al. (1982), Burns et al. (1985), and Sudman et al. (1987), karyotypes were used to identify individuals as either parentals (*majusculus*, 2n = 70 and FN = 68; *lutescens*, 2n = 72 and FN = 86–98; or *halli*, 2n = 72 and FN = 70) or hybrids. Because of the potentially complex karyotypes formed as a result of hybridization no attempt was made to distinguish among F_2 and backcross karyotypes. These complex karyotypes were designated simply as a ''hybrid karyotype.''

Morphology and pelage coloration.—Condylobasal length was determined from adult specimens using dial calipers. Adult age classes were determined following Heaney and Timm (1985) using a combination of pelage coloration, closure of cranial sutures, and development of cranial crests. Measurements were recorded in millimeters to the nearest one-hundredth. A Student's *t*-test was used to determine if significant differences were present among individuals relevant to the condylobasal length within each population.

Pelage coloration of adult specimens was determined by assessing degree of lightness or darkness of the dorsal hair. Specimens were separated into 1 of 5 groups with group 1 representing the lightest coat color and group 5 representing the darkest coloration. A Student's *t*-test was used to determine if significant differences were present among individuals relevant to the pelage coloration within each population.

Construction of genotypes.—The 3 data sets (*Cytb*, interphotoreceptor retinoid-binding protein gene, and chromosomes) were used in combination to develop genotypes for all individuals. Results are reported by locality in Table 1.

Results

Results of the chromosomal, mitochondrial DNA, and nuclear DNA analyses (Table 1) are addressed below by locality. An overall genotype was determined from the combination of the 3 data sets and was used as the final indicator for hybridization and directionality of hybridization.

Tilden locality (n = 3).—Two individuals possessed the karyotype corresponding to *G. b. majusculus* and 1 individual displayed the karyotype typical of *G. lutescens*. Based on the nuclear *Rbp*3 marker, 1 individual was identified as *G. lutescens*, 1 as *G. b. majusculus*, and 1 as a hybrid (heterozygous for alleles of *G. lutescens* and *G. b. majusculus*). All 3 individuals possessed the *Cytb* pattern associated with *G. b. majusculus*. Using results obtained from the 3 data sets, 2 individuals were hybrids: both possessed a greater percentage of the genome of *G. lutescens* but possessed the *Cytb* pattern characteristic of *G. b. majusculus*. One individual was identified as a pure *G. b. majusculus*.

Meadow Grove locality (n = 2).—Two individuals possessed the karyotype, *Cytb* pattern, and *Rbp*3 corresponding to *G. b. majusculus*.

Neligh locality (n = 5).—All individuals possessed the karyotype corresponding to *G. lutescens*. Based on the nuclear *Rbp*3 marker, 3 individuals were identified as *G. lutescens*, 1 as *G. b. majusculus*, and 1 as a hybrid. Three individuals possessed the *Cytb* pattern associated with *G. b. majusculus* and 2 individuals possessed *G. lutescens*–like *Cytb*. Results from the 3 data sets indicated that 4 individuals were hybrids: 3 possessed a greater percentage of the genome of *G. lutescens* and 1 hybrid possessed a greater percentage of the genome of *G. b. majusculus*. One individual was a pure *G. lutescens*.

Oakdale locality (n = 29).—Six individuals possessed the karyotype corresponding to *G. lutescens*, 18 possessed the karyotype predicted for hybrids, no *G. b. majusculus*–like karyotypes were detected, and 5 did not produce scorable karyotypes. Based on the nuclear *Rbp*3 marker, 19 individuals were identified as *G. lutescens*, 7 as *G. b. majusculus*, and 3 as hybrids. Twenty-three individuals possessed the *Cytb* pattern associated with *G. b. majusculus* and 6 individuals possessed *G. lutescens*–like *Cytb*. Based on the 3 data sets, 24 individuals were identified as hybrids, 18 of which possessed a greater percentage of the genome of *G. lutescens* and 6 possessed a greater percentage of the genome of *G. b. majusculus*; however, 22 of 24 hybrids had *G. b. majusculus*–like *Cytb* and only 2 hybrids had *G. lutescens*–like *Cytb*. Four individuals were identified as pure *G. lutescens* and 1 individual was a pure *G. b. majusculus*.

Sutherland locality (n = 28).—Twenty-one individuals possessed the karyotype corresponding to *G. j. halli*, 6 possessed the karyotype predicted for *G. lutescens*, and 1 did not produce a scorable karyotype. Based on the nuclear *Rbp*3 marker, 22 individuals were identified as *G. j. halli*, 5 as *G. lutescens*, and 1 as a hybrid. Twenty-three individuals possessed the *Cytb* pattern associated with *G. j. halli* and 5 individuals possessed *G. lutescens*–like *Cytb*. Based on the 3 data sets, 1 individual was identified as a hybrid and possessed a greater percentage of the genome of *G. lutescens*; however, this individual had *G. j. halli*–like *Cytb*. Twenty-two individuals were pure *G. j. halli* and 5 individuals were pure *G. lutescens*.

Maxwell locality (n = 9).—One individual possessed the karyotype corresponding to *G. j. halli*, 2 possessed the karyotype associated with *G. lutescens*, 3 possessed karyotypes predicted for hybrid individuals, and 3 did not produce scorable karyotypes. Based on the nuclear *Rbp*3 marker, 4 individuals were identified as *G. j. halli*, 4 as *G. lutescens*, and 1 as a hybrid. Seven individuals possessed the *Cytb* pattern associated with *G. j. halli* and 2 individuals possessed *G. lutescens*–like *Cytb*. Based on the 3 data sets, 5 individuals were identified as hybrids: 4 possessed a greater percentage of the genome of *G. lutescens* and 1 possessed a greater percentage of the genome of *G. j. halli*; however, 4 of 5 hybrids had *G. j. halli*–like *Cytb* and only 1 hybrid had *G. lutescens*–like *Cytb*. Three individuals were pure *G. j. halli* and 1 individual was a pure *G. lutescens*.

Condylobasal length and pelage coloration were recorded for each specimen (Table 1). In the Oakdale hybrid zone, the average condylobasal length and pelage scores for *G. b. majusculus* ($n = 3$), hybrids ($n = 25$), and *G. lutescens* ($n = 3$) were 54.42 mm and 4.0, 43.46 mm and 2.2, and 39.52 mm and 1.3, respectively. Specimens of *G. b. majusculus* were significantly different from hybrids and *G. lutescens* ($P = 0.046$ and $P = 0.02$) in condylobasal length. No significant differences were found in pelage coloration between the 3 groups. In the Sutherland hybrid zone, the average condylobasal length and pelage scores for *G. lutescens* ($n = 3$), hybrids ($n = 5$), and *G. j. halli* ($n = 21$) were 43.22 mm and 1.7, 43.30 mm and 2.0, and 42.61 mm and 3.1, respectively. No significant differences were found in condylobasal length or pelage coloration among any of the 3 groups.

Discussion

Genetic data (chromosomes, *Cytb* sequences, and *Rbp*3 sequences) depicted 2 areas of hybridization between taxa of *Geomys* in Nebraska. The 1st of these corresponded to Oakdale, Antelope County, and vicinity in northeastern Nebraska, and the 2nd corresponded to Lincoln County (Maxwell and Sutherland) in southwestern Nebraska.

The Oakdale hybrid zone initially described by Timm et al. (1982), Heaney and Timm (1983, 1985), and Burns et al. (1985) appears to be the result of an ecotone (Endler 1977) formed as sandy soils from west-central Nebraska meet the more endurate soils from northeastern Nebraska (Fig. 2). The 2 species of pocket gophers in the region show an affinity to soil type, with *G. lutescens* inhabiting the sandy soil to the west and *G. b. majusculus* preferring the harder soils characteristic of eastern Nebraska and north along the valley of the Elkhorn River. The ecotone near Oakdale produces an interdigitation of soil types and allows for potential hybridization of the 2 pocket gopher species. Pocket gophers collected from 4 localities near Oakdale (Neligh, Oakdale, Tilden, and Meadow Grove) document the distribution of genotypes and species. In general, specimens corresponding to pure genotypes of *G. lutescens* were restricted to the more western localities (Neligh and Oakdale), whereas specimens representing pure genotypes of *G. b. majusculus* were found in the more eastern localities (Meadow Grove and Tilden). However, 1 pure genotypes of *G. b. majusculus* was found at the Oakdale locality. Although Heaney and Timm (1983, 1985) reported that introgression in this region was inconsequential, 30 of the 39 specimens collected in this area possessed a hybrid genotype. Although sample sizes were small, hybridization exceeded 60% at 3 of the localities.

In the Lincoln County (Maxwell and Sutherland) hybrid zone, distributions of *G. lutescens* and *G. j. halli* are determined by the Platte River complex (Fig. 3). Typically, *G. lutescens* occurs north of the Platte River and *G. j. halli* occurs to the south; however, oxbows and islands often result in 1 of the species being placed on the ''wrong side'' of the Platte River. In these instances, individuals of *G. lutescens* and *G. j. halli* may come into contact and hybridize. In this study, 28 specimens were collected in the vicinity of the North Platte and South Platte rivers near Sutherland and 9 individuals were collected from north and south of the Platte River near Maxwell. At the Sutherland locality, 5 individuals representing pure genotypes of *G. lutescens*, 22 individuals representing pure genotypes of *G. j. halli*, and 1 hybrid individual were collected. Although both parental types were collected in close proximity, the presence of a single hybrid individual reflects a low level of hybridization. Interestingly, all individuals collected south of the North Platte River (21 individuals between North and South Platte rivers and 1 individual south of the South Platte River; Fig. 3) appeared to be pure *G. j. halli*. However, individuals collected north of the North Platte River were either pure *G. lutescens* ($n = 5$) or a hybrid ($n = 1$). At the Maxwell locality, 5 of 9 specimens were hybrids, 1 was pure *G. lutescens*, and 3 were pure *G. j. halli*, indicating a much higher level of hybridization than at Sutherland. At this time, it is unclear why the 2 localities possess different levels of hybridization. Similar to the situation at Sutherland, individuals collected at Maxwell north of the Platte River were either pure *G. lutescens* ($n = 1$) or hybrids ($n = 5$), whereas specimens from south of the Platte were pure *G. j. halli* ($n = 3$).

Based on observations from these 2 localities, it would appear that the Platte River complex is a barrier to *G. lutescens* moving southward but is not a barrier to northward movement of *G. j. halli*. The primary difference between Maxwell and Sutherland is that there is a single river at Maxwell (Platte River) and 2 rivers (North Platte and South Platte rivers) at Sutherland. Alternatively, the rivers may only be incidental to the separation of these 2 taxa in Lincoln County. At both Sutherland and Maxwell, the Nebraska Sand Hills approach the north sides of the rivers. North of Sutherland, the deep eolian soils of the Sand Hills intrude on the North Platte River, with some of the fine wind-blown sands even overlaying the coarse river sands at a few points on the south bank of the river, leaving no river valley north of the river. North of Maxwell, the river valley is at least 1.5 km wide before reaching the Sand Hills. In the presence of other species of pocket gophers, *G. lutescens* appears to be confined to the fine, poorly compacted soils of the Sand Hills. Therefore, the differences in the rates of hybridization between the 2 areas may simply reflect the greater opportunity for the 2 species to come into contact near Maxwell than at Sutherland.

Results of mitochondrial analyses from the 2 hybrid zones provide some insight into the hybridization process. At Oakdale, 27 of 30 hybrid individuals had mitochondrial DNA representing *G. b. majusculus*. This indicates that the genetic history of these individuals involved a female *G. b. majusculus* mating with a male *G. lutescens*. Given that most of the hybrids identified in this study were either F_2 or multigeneration backcrosses, this event may have taken place ≥ 1 generations in the past. Conversely, at the Lincoln County hybrid zones, 5 of 6 hybrid individuals had mitochondrial DNA representing *G. j. halli*, indicating that the crosses involved a female *G. j. halli* mating with a male *G. lutescens*.

Morphologic analyses revealed that specimens of *G. b. majusculus* were significantly larger than *G. lutescens* and hybrids. In general, specimens of *G. b. majusculus* were darker

in pelage coloration ($\bar{X} = 4.0$) than hybrids ($\bar{X} = 2.2$) or *G. lutescens* ($\bar{X} = 1.3$), although this difference was not statistically significant. Comparisons of *G. lutescens*, *G. j. halli*, and hybrids did not detect any significant differences in size or pelage color, although *G. j. halli* appeared to be slightly darker than *G. lutescens* ($\bar{X} = 3.1$ and $\bar{X} = 1.7$, respectively). The lack of statistically supported groups (parental types and hybrids) disagrees with the findings of Heaney and Timm (1985); however, our study was hampered by small sample sizes of parental types.

The results of this and other studies (Burns et al. 1985; Heaney and Timm 1983, 1985) show varying levels of hybridization between *G. lutescens* and *G. b. majusculus* at the Oakdale hybrid zone. In addition, the data from the Sutherland and Maxwell localities revealed hybridization between *G. lutescens* and *G. j. halli*. Obviously, the question at hand is what impact (if any) does hybridization have on the recognition of species? We approach this question in 2 scenarios.

First, both contact zones appear to be the result of secondary contact produced by location of soil types. *G. lutescens* is a specialist, occupying the deep sandy soils of the Nebraska Sand Hills complex. The Sand Hills region is large (approximately the size of the Sahara Desert of Africa) and has had open dunes at times during the last 1,500 years. *G. b. majusculus*, on the other hand, is a generalist and occupies the more endurate soils along riparian habitats and deep loamy soils to the east. At Oakdale, prevailing westerly winds have allowed the pale, small-grained sands to encroach upon the endurate soils to the east. This scenario produces the secondary contact between 2 taxa that previously were allopatric, leading to levels of hybridization >60% at Oakdale. At the Sutherland and Maxwell sites hybridization occurs, but at a lower frequency (6 of 37) and appears to result from the movement of wind-blown soils similar to that at Oakdale. At Sutherland and Maxwell, *G. j. halli* occupied the mixed and endurate soils to the south, whereas *G. lutescens* occupied the wind-blown sandy soils to the north.

At both hybrid zones, the majority of hybrid individuals (27 of 30 at Oakdale and 4 of 5 at Sutherland and Maxwell) possessed mitochondrial genomes affiliated with the specialist form (*G. lutescens*). This follows the predictions of the Kaneshiro hypothesis (Kaneshiro 1976, 1980) as reported in other species of *Geomys* by Baker et al. (1989), Bradley et al. (1991), and Jones et al. (1995). The unidirectional pattern of mate selection implies the operation of pre- or postmating isolating mechanisms during hybridization.

Second, the genetic species concept of Baker and Bradley (2006) addresses hybridization and its impact upon speciation. The dogma associated with the biological species concept (sensu Mayr 1942) associates hybridization with genetic introgression and consequently a breakdown in reproductive isolation. Baker and Bradley (2006) argued that hybridization has little or no impact on genetic introgression as long as the hybrid zones are small and that the integrity of the respective gene pools (genetic isolation) is maintained across the respective species distribution. In fact, Baker and Bradley (2006) predict that hybrid zones will be common between genetically isolated species of mammals. Consequently, more studies are needed to measure levels of introgression away from the center of each hybrid zone.

Our study supports the taxonomic recommendations of Sudman et al. (2006), who used genetic divergence, reciprocal monophyly, and summarized existing chromosome and allozyme data in recognizing 3 taxa of pocket gophers in these areas of Nebraska—specifically, *G. bursarius majusculus* in eastern Nebraska, *G. lutescens* in the Sand Hills and adjacent areas of central and western Nebraska, and *G. jugossicularis halli* in southwestern Nebraska. However, the exact distribution and relationships of these species within Nebraska and the surrounding states are yet to be determined in detail.

Geomys bursarius majusculus with its type locality at Lincoln, Lancaster County, Nebraska, was recognized by Hall (1981), but was treated as a junior synonym of the nominate subspecies with a restricted type locality of Elk River, Sherburne County, Minnesota, by Heaney and Timm (1983). We continue to recognize *G. b. majusculus* because there appears to be more variation at both the morphometric and molecular levels (Elrod et al. 2000; Jolley et al. 2000; Sudman et al. 1987, 2006) than would be expected among populations assigned to a single subspecies (*G. b. bursarius*) as arranged by Heaney and Timm (1983). Hall (1981) mapped the distribution of *G. b. majusculus* in the eastern one-third of Nebraska, throughout Iowa, in northern Missouri, and northeastern Kansas.

Our study supports recognition of *G. lutescens* as a monotypic species occupying the deep sandy soils of the Nebraska Sand Hills. In the absence of other species of *Geomys*, *G. lutescens* also is found in areas adjacent to the Sand Hills in the Pine Ridge and North Platte Valley of western Nebraska and into eastern Wyoming and southern South Dakota. This makes *G. lutescens* the only mammalian species with its primary geographic distribution centered on the Nebraska Sand Hills region. East of Lincoln County, Sudman et al. (1987:527–528) found *G. lutescens* to be separated from *G. j. halli* "by a 40-km wide zone of heavy loessal soil on the north side of the Platte River." It was only in this area in extreme southern Buffalo County that Sudman et al. (1987) trapped *G. j. halli* on the north side of the Platte River. The distributions of these 2 taxa west of Lincoln County in the southern Nebraska Panhandle and northeastern Colorado have not been studied.

The name combination *Geomys jugossicularis halli* was 1st used by Sudman et al. (2006). This taxon's type locality is near Ellis, Ellis County, Kansas, and it occurs in southwestern Nebraska, northwestern Kansas, and adjacent parts of Colorado. However, its distributional boundaries (except to the north) are still undetermined, as is its relationship to the nominate subspecies. Hendricksen (1973:364) studied a transect of populations of *Geomys* from central Colorado to eastern Kansas in which the contact zone between eastern and western populations "appears to be quite narrow (less than 20 miles)" in Osborne County, Kansas. Burns et al. (1985:113) later intensively studied this zone and found "no evidence of intergradation" in populations that we would assign to *G. j. halli* and *G. b. majusculus* in Smith and Osborne counties. In Smith County, Burns et al. (1985:113) found populations of

these species ''within 5 km of each other,'' but no overlap of populations was demonstrated. A similar situation is expected, but has not been found, to the north in Adams, Franklin, Kearney, and Webster counties in southern Nebraska. In Kansas and eastern Colorado, *G. j. halli* and *G. j. jugossicularis* would be expected to intergrade between the Smoky Hill and Arkansas rivers, but pocket gophers from this area have not been studied in detail with morphometric and molecular techniques. Sudman et al. (2006) demonstrated based on molecular data that branching patterns and genetic distances support placing the taxa *jugossicularis* and *halli* into the species *G. jugossicularis* and placing *majusculus*, *industrius*, and *major* into the species *G. bursarius*. However, the zones of contact among these taxa in southwestern Kansas and the panhandles of Oklahoma and Texas have yet to be located.

With molecular data (Sudman et al. 2006) indicating that as many as 6 species of *Geomys* are represented in the taxon previously considered to be a single species, *G. bursarius*, it is obvious that additional studies are needed to investigate relationships among these taxa. Clearly, more intensive field investigations using the full range of modern systematic techniques are needed to study the numerous new contact zones created by this revised systematic arrangement.

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