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Distribution, Habitat Use, and Identification of Masked Shrews, Sorex cinereus and Sorex haydeni, in Minnesota

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ABSTRACT.—Several studies support specific status for the prairie form of *Sorex cinereus,* designated as *Sorex haydeni*. Evidence for introgression between these species has been found in Alberta despite significant sequence differences (>15%) in mitochondrial DNA. We identified to species 94 masked shrews using mtDNA and morphological criteria to assess the distribution of these two species in Minnesota and to examine the extent of introgression in zones of sympatry. Only four specimens scored genetically and morphologically as *S. haydeni*, indicating its restricted distribution. Four specimens had incongruent genetic and morphological identifications, suggesting introgression between the two species.

INTRODUCTION

Field studies of masked shrews (*Sorex cinereus*) in Minnesota have yielded specimens from essentially every county (Hazard, 1982) in both forested and grassland habitats (Tester and Marshall, 1961; Iverson *et al.*, 1967). The species was considered to be a widely distributed

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ecological generalist with the subspecies *S. c. haydeni* restricted to prairies. However, van Zyll de Jong's (1976, 1980) studies of geographic variation in morphological characters suggested that the prairie form of the masked shrew was specifically distinct from the woodland form, and he assigned the prairie form to *Sorex haydeni*.

Most subsequent research has supported specific status for *S. haydeni*. Studies of allozyme variation suggested to George (1988) that *S. cinereus* and *S. haydeni* were related very closely, probably last sharing a common ancestor in the Wisconsinan (~11,000 years ago). Stewart *et al.* (1993), however, were unable to discriminate between *S. cinereus* and *S. haydeni* using only allozyme data. Volobouev and van Zyll de Jong (1994) found the two to be distinct chromosomally, differing in both 2n (64 in *S. haydeni* and 66 in *S. cinereus*) and FN numbers (66 in *S. haydeni* and 70 in *S. cinereus*). Comparisons of mitochondrial DNA (mtDNA) sequences (Stewart and Baker, 1997) indicated that *S. haydeni* and *S. cinereus* are quite distinct on the basis of a large sequence difference (>15%). In addition, *S. haydeni* has an insertion that is absent in *S. cinereus*. Despite mtDNA differences, Stewart and Baker (1997) suggest introgression between the species in Alberta, resulting in some individuals morphologically characteristic of *S. haydeni* possessing haplotypes of *S. cinereus*. Van Zyll de Jong and Kirkland (1989) conducted morphological studies of the two taxa in eastern and central U.S. Based on significant divergence of cranial measurements, they concluded there was no evidence of intergradation in a large area of geographic overlap encompassing most of the original grassland portion of western Minnesota, Iowa and some prairie portions of Canada. However, the number of specimens from several Minnesota counties included in the study was low (approximately half of the counties were represented by a single specimen), and lack of mtDNA data obscured putative hybrids.

In light of the findings by Stewart and Baker (1997), we used specimens trapped in western Minnesota to re-examine the distribution of *S. cinereus* and *S. haydeni* and the possibility of intergradation. We analyzed mtDNA sequences for 94 shrews and independently identified specimens to species using morphological criteria (van Zyll de Jong, 1980). We then merged the data to reveal the distribution of *S. cinereus* and *S. haydeni* mitochondrial genomes and the concordance between mtDNA and morphological species-level identification. The latter allowed us indirectly to measure the extent of introgression between *S. cinereus* and *S. haydeni* and to assess the use of morphological characteristics to distinguish between the two species.

METHODS

Field collection.—Forty-two specimens were trapped in 1999 by ECB, AKB and KMK at 23 localities covering 10 western counties. Three traplines of approximately 30 traps each were set for one night at each location. At each trap locality we recorded data on habitat type. All specimens were prepared in the field and tissue samples preserved in DMSO buffer. An additional 52 specimens were provided by the 1998 and 1999 Minnesota County Biological Survey (MCBS; G. Nordquist), from 11 other counties. Figure 1 shows the counties where specimens were collected in 1998 and 1999 as well as localities of 363 specimens housed in the Bell Museum of Natural History. The museum specimens were identified to species using morphological criteria but we did not obtain genetic information.

Morphological assessment.—We scored all 94 specimens for position of the infraorbital and lacrimal foramina, pigmentation pattern of the lower anterior tooth row, color of the tail tuft, and field-recorded length of the tail, as described by van Zyll de Jong (1980). Ultimately, tail length was excluded from the analysis because measurements were highly variable preventing a

clear delineation between the two species. Variation in tail measurements is often high due to differences in measurement techniques. Van Zyll de Jong (1980) also found wide ranges and considerable overlap between the tail lengths of the two species (30.0–46.0 mm, *S. cinereus*; 25.0–38.0 mm, *S. haydeni*). The scores for the first three characters were discrete and recorded as *C* for *S. cinereus*-like phenotype, *H* for *S. haydeni*-like phenotype, and *I* for an intermediate phenotype. Using the scores for pigmentation, foramina position and tail tuft color, we identified a specimen as "pure *S. cinereus*" if it received all *C* or a combination of *C* and *I* scores or as "pure *S. haydeni*" if it received all *H* or a combination of *H* and *I* scores. Any specimen that received a combination of *C* and *H* scores we designated as "intermediate."

We could not score pigmentation of eight specimens due to tooth wear and 13 specimens were missing the tail tuft. However, with the exception of six specimens, all identifications were based on at least two scores. The exceptions were included in the dataset because all scored morphologically and genetically as *S. cinereus*. To ensure that variation in symmetry of the cranial characters would not affect the scoring of the specimens, we took a random sample of 30 specimens and scored the position of the left and right infraorbital and lacrimal foramina. Only in two of these specimens were the left and right scores different, and the discrepancy was between an intermediate and a *cinereus* score. We feel confident bilateral asymmetry did not affect the morphological scoring of the specimens.

Analysis of mtDNA.—We isolated DNA from tissue using standard Chelex methods. Based on the work of Stewart and Baker (1997) we designed shrew-specific primers to amplify a piece of approximately 150 base pairs of the mitochondrial DNA control region, in which *S. haydeni* and *S. cinereus* differ by over 15% in base sequence as well as the insertion mentioned

previously. We used the same primers for direct sequencing using an ABI 310 automated sequencer. We scored each individual for the presence and absence of the *haydeni* insert.

RESULTS

Seventy-one of the 94 (76%) study specimens scored morphologically as pure *S. cinereus*. Specimens MMNH 18471 (Becker Co.), 18474 (Stevens), 18475 (Stevens), 18476 (Yellow Medicine), 18492 (Redwood), 18497 (Renville) and 18542 (Brown) were scored as pure *S. haydeni* and the remaining specimens (16) scored as intermediate.

Table 1 summarizes the morphological and mtDNA identifications of the study specimens. Eight of the 94 individuals had the mtDNA insertion typical of *S. haydeni*. These were specimens MMNH 17940 (Kittson Co.), 17941 (Kittson), 17957 (Polk), 18464 (Swift), 18471 (Becker), 18474 (Stevens), 18475 (Stevens) and 18476 (Yellow Medicine). Four of these specimens scored morphologically as *S. haydeni* (18471, 18474, 18475 and 18476) and one (17940) scored as intermediate, with *S. haydeni*-like tooth pigmentation and color of tail tuft and *S. cinereus*-like position of the infraorbital and lacrimal foramina. The remaining three specimens were identified morphologically as *S. cinereus*. Of the 81 specimens with mitochondrial genome of *S. cinereus*, 66 scored morphologically as *S. cinereus*, 14 scored as intermediates, and one scored as pure *S. haydeni* (18542). MMNH 17941, 17957, 18464 and 18542 had incongruent morphological and genetic identifications and were caught in Kittson, Polk, Swift and Brown counties, respectively. Finally, three specimens genetically identified as *S. cinereus*, showed a deletion. This deletion has been reported previously in *S. cinereus* (Stewart and Baker, 1994, 1997). Morphologically, two of the specimens with deletions scored

as pure *S. cinereus* and one scored as an intermediate with *S. haydeni*-like tooth pigmentation and *S. cinereus*-like tail tuft and position of foramina.

DISCUSSION

Although our trapping efforts focused on open grasslands, which are areas of potential overlap, based on study, MCBS, and museum specimens, the distribution we observed for these two species matches well with that observed by van Zyll de Jong and Kirkland (1989). *Sorex haydeni* appears to be relatively rare in Minnesota and restricted to counties where prairie and grassland habitat predominate, whereas *S. cinereus* extends across the state occupying wooded and open habitat (Fig. 1). In addition, as van Zyll de Jong and Kirkland (1989) did, we found a broad-scale overlap of distributions for the two species. In every county where we captured *S. haydeni* (as identified morphologically and genetically), we captured *S. cinereus* also. We recognize that within each county, *S. cinereus* and *S. haydeni* populations might be segregated, however, the evidence we found for introgression suggests that in some areas the two species come into contact at least to breed.

Our results differ from those of van Zyll de Jong and Kirkland (1989) in that we found evidence of introgression. For four specimens the genetic and morphological identifications did not agree and 16 specimens scored as morphological intermediates, having characters of both *S. cinereus* and *S. haydeni*. It is possible that the disagreement of our data with those of van Zyll de Jong and Kirkland's study resulted from a difference in sample sizes. The introgression that Stewart and Baker (1997) report for Alberta resulted in individuals with the phenotype of *S. haydeni* and mtDNA of *S. cinereus*. The introgression we found was mostly the opposite. One specimen (18542) showed the introgression pattern observed by Stewart and Baker (1997), but

the remaining three were morphologically identified as *S. cinereus* and possessed mtDNA of *S. haydeni*. This suggests that hybridization can occur between males and females of both species.

The bilateral asymmetry observed by Foresman and Jensen (1992) in Montana, which could affect accurate identification of specimens, was essentially absent in our samples. Van Zyll de Jong and Kirkland (1989) also found the Montana population to be divergent from the remaining U.S. samples included in their study. Results indicated a smaller size and shorter unicuspid length in the *S. cinereus* population. These anomalies appear to be characteristics only of the Montana population. However, using cranial characters and tail tuft color we were unable to identify 20 specimens accurately. Four of these specimens had incongruent genetic and morphological identifications and the remaining 16 (intermediate specimens) showed a combination of *S. cinereus*-like and *S. haydeni*-like phenologies. We believe these specimens to be evidence of potential introgression, which may obscure species boundaries.

We present evidence for a zone of introgression in Minnesota. The exact extent of this zone remains to be determined, yet with results from Stewart and Baker (1997), it might span Alberta, Minnesota and Iowa. This possibility raises the questions of what effect this intergradation will have on the population of the apparently rare *S. haydeni* and what can be done to mitigate negative effects.

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TABLE 1.—Summary of morphological scores and mtDNA results for *Sorex cinereus* and *S. haydeni*

	mtDNA Results		
Morphology	*S. cinereus*	*S. haydeni*	Unknown
S. cinereus	66	3	2
S. haydeni	1	4	2
Intermediate	14	1	1

TABLE 2.—Habitat preferences of 82 shrews captured in 1998 and 1999. Values represent number of individuals captured in each habitat category based on genetic and morphological (in parentheses) data

Habitat type	*Sorex cinereus*	*Sorex haydeni*	Morphological intermediate
Restored prairie	11 (9)	2 (0)	4
Mesic prairie	8 (8)	3 (3)	1
Dry prairie	10 (7)		4
Disturbed prairie	3 (4)		1
Mesic grassland	7 (7)	1 (1)	
Dry grassland	3 (2)		1
Pasture	1 (1)		
Road ditch	10 (9)	1 (0)	2
Old field	15 (14)	0 (2)	2
Canary grasses	1 (1)		
Maple-basswood forest	2 (2)		
	1 (1)		
Forest clearing	1 (1)		
Savannah	1 (1)		
Rock outcrop			

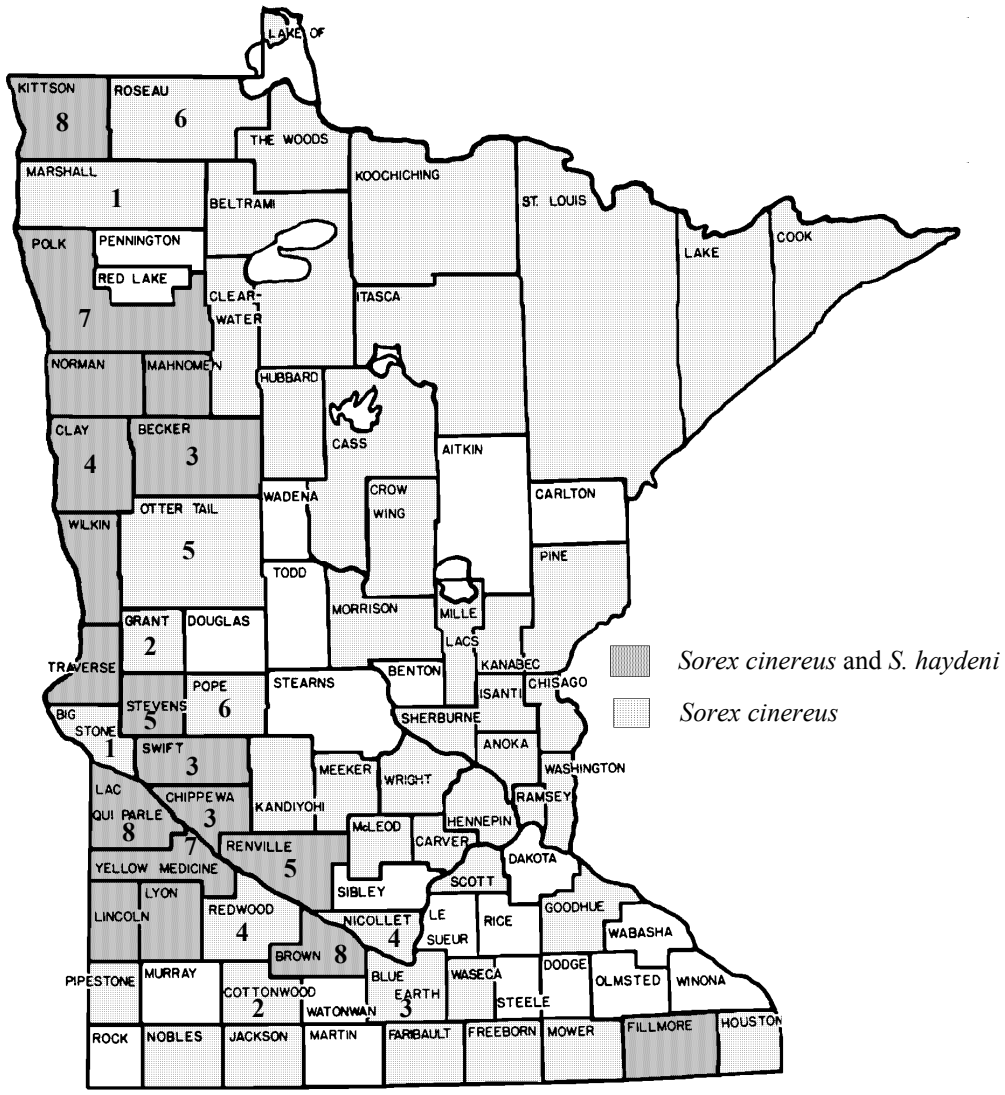
LAKE OF
KITTSON
8
ROSEAU
6
THE WOODS
MARSHALL
1
BELTRAMI
KOOCHICHING
ST. LOUIS
LAKE
COOK
POLK
PENNINGTON
RED LAKE
7
CLEAR-
WATER
ITASCA
NORMAN
MAHNOMEN
HUBBARD
CLAY
4
BECKER
3
CASS
AITKIN
WADENA
CROW
WING
CARLTON
WILKIN
OTTER TAIL
5
TODD
PINE
MORRISON
MILLE
LACS
GRANT
2
DOUGLAS
TRAVERSE
BENTON
KANABEC
STEARNS
ISANTI
CHISAGO
POPE
6
STEVENS
5
BIG
STONE
1
SHERBURNE
ANOKA
SWIFT
3
MEEKER
WRIGHT
WASHINGTON
LAC
QUI PARLE
8
CHIPPEWA
3
KANDIYOHI
RAMSEY
McLEOD
HENNEPIN
7
CARVER
RENVILLE
5
DAKOTA
YELLOW MEDICINE
SIBLEY
SCOTT
LYON
LINCOLN
REDWOOD
4
NICOLLET
4
LE
SUEUR
RICE
GOODHUE
WABASHA
BROWN
8
DODGE
PIPESTONE
MURRAY
BLUE
EARTH
3
WASECA
OLMSTED
WINONA
COTTONWOOD
2
WATONWAN
STEELE
ROCK
NOBLES
JACKSON
MARTIN
FARIBAULT
FREEBORN
MOWER
FILLMORE
HOUSTON
Sorex cinereus and S. haydeni
Sorex cinereus

FIG. 1.—Counties where *Sorex cinereus* and *S. haydeni* have been trapped based on 94 specimens captured in 1998 and 1999 and 363 specimens housed in the Bell Museum of Natural History. Numbers indicate sample sizes of shrews from each county for which both morphological and genetic data were obtained. The four specimens with incongruent identifications were captured in Kittson, Polk, Swift and Brown counties. Adapted from Hazard, 1982