Genetic Variation Within and Among Populations of Delmarva Fox Squirrels (*Sciurus niger cinereus*)

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Abstract

We compared genetic variation within and among a total of 19 populations of fox squirrels (Sciurus niger) using data from horizontal starch-gel electrophoresis. The primary focus of this study was populations of Delmarva fox squirrels (S. n. cinereus) from the Eastern Shore of Maryland and Virginia (Blackwater and Chincoteague National Wildlife Refuges) as well as other populations of eastern fox squirrels from Maryland, Virginia, and Georgia. Overall variation in Delmarva fox squirrel populations from Blackwater and Chincoteague National Wildlife Refuges is comparable to that found in populations of other eastern fox squirrels and that reported by Moncrief (1987) for western populations. Additionally, these two S. n. cinereus populations possess an electrophoretically detectable genetic attribute not present in any other population of S. niger examined to date. This information on genetic variation should be incorporated into management plans and strategies for continued reintroduction of S. n. cinereus throughout the Delmarva Peninsula. Although our findings are encouraging, in that there is genetic variation in S. n. cinereus, the long-term security and sustainability of Delmarva fox squirrel populations remains uncertain.

Introduction

The U.S. Fish and Wildlife Service recovery plan for the endangered Delmarva fox squirrel (Sciurus niger cinereus) seeks to reintroduce populations throughout the former range of this subspecies. As of May, 1987, squirrels had been transplanted to 12 unoccupied sites in Maryland, Pennsylvania, and Delaware, as well as two sites in Virginia. Dueser and Terwilliger (1987) identified several biological and ecological constraints that should affect future attempts to reintroduce S. n. cinereus. As they emphasized, the genetic makeup of source and introduced populations should be considered in developing a comprehensive protocol for continued reintroduction and long-term management of populations of the Delmarva fox squirrel. To date, the only study of electrophoretic variation in S. n. cinereus has been Morgan and Quattro's (1986) analysis of 57 individuals from Eastern Neck Island National Wildlife Refuge and Dorchester County, Maryland. This investigation reported no electrophoretically detectable variation at 23 loci scored from blood.

To confirm or deny the total lack of genetic variation reported by Morgan and Quattro (1986), we conducted a more thorough electrophoretic analysis of Delmarva fox squirrels, assaying proteins from tissue extracts as well as blood. We also included several other populations of eastern fox squirrels in

our analyses and incorporated genetic information available for western populations of fox squirrels from Moncrief's (1987) dissertation study. The objective of this study was to provide important information about genetic variation in populations of S. n. cinereus in the context of a more general (and hence more informative) survey of electrophoretic variation in other populations of fox squirrels.

Materials and Methods

Fox squirrels (Sciurus niger) are one of the most geographically variable mammals in North America; ten subspecies are currently recognized based on sometimes striking variation in size and coat coloration (Fig. 1; Hall, 1981). This dramatic variation among subspecies must be considered when analyzing variation within subspecies.

For this study, we analyzed a total of 192 individuals, representing 10 samples (Fig. 2) from the following localities (sample sizes are indicated in parentheses and represent tissue extracts unless noted otherwise): 1) Maryland: Dorchester Co., Blackwater National Wildlife Refuge (34); 2) Virginia: Accomack Co., Chincoteague National Wildlife Refuge (40 blood, 6 tissues); 3) Virginia: Alleghany, Augusta, Botetourt, Craig, Giles, Highland, Rockbridge, Rockingham, Russell, Shenandoah, Smyth, Washington, and Wythe Cos. (40); 4) Maryland: Allegany Co. (40);

5) Georgia: Jasper and Jones Cos., Piedmont National Wildlife Refuge (40); 6) Mississippi: Holmes Co. (4); 7) Louisiana: East Baton Rouge Par. (1); 8) Louisiana: Vernon Par. (1); 9) Louisiana: Bossier Par. (1); 10) TX: Atascosa Co. (1). Voucher specimens for all individuals in samples 1-5 are deposited in the Virginia Museum of Natural History, Martinsville, VA. S. n. cinereus individuals were transported and are housed under Regional Blanket Permit #697823, issued by the U.S. Fish and Wildlife Service to NDM. Blood was obtained from live-captured S. n. cinereus individuals during annual nest-box checks with permission from and cooperation of the Virginia Department of Game and Inland Fisheries and the U.S. Fish and Wildlife Service.

Protein variation was determined using horizontal starch-gel protein electrophoresis. Techniques for tissue preparation and staining followed Harris and Hopkinson (1976), Moncrief (1987), Murphy et al. (1990), and Selander et al. (1971). Heart, liver, kidney, and skeletal muscle tissues were available from all populations; blood was available only from the Chincoteague National Wildlife Refuge population. Many proteins can be detected in both blood and tissue extracts; however, some enzymes are absent from blood, and some are absent from or not easily detected in tissue extracts.

We analyzed 34 protein systems that are encoded by 41 presumptive gene loci. The 34 proteins (33 enzymes and the transport protein hemoglobin, Hb) analyzed were: acid phosphatase (ACP, Enzyme Commission No. 3.1.3.2), aconitase (ACN-1, 4.2.1.3),

adenylate kinase (AK, 2.7.4.3), alcohol dehydrogenase (ADH, 1.1.1.1), creatine kinase (CK, 2.7.3.2), diaphorase (DIA, 1.6.1.1), erythrocytic acid phosphatase (EAP, 3.1.3.2), fumarase (FUM, 4.2.1.2), glucose-6-phosphate dehydrogenase (G6PD, 1.1.1.49), glutamate dehydrogenase (GLUD, 1.4.1.-), glutamate oxaloacetate transaminase (GOT-1,-2, 2.6.1.1), glutathione reductase (GR, 1.6.4.2), glycerol-3-phosphate dehydrogenase (G3PD, 1.1.1.8), guanine deaminase (GDA, 3.5.4.3), hexokinase (HK, 2.7.1.1), hemoglobin (Hb), isocitrate dehydrogenase (IDH-1,-2, 1.1.1.42), lactate dehydrogenase (LDH-1,-2, 1.1.1.27), malate dehydrogenase (MDH-1,-2, 1.1.1.37), malic enzyme (ME, 1.1.1.40), mannose phosphate isomerase (MPI, 5.3.1.8), nucleoside phosphorylase (NP, 2.4.2.1), octanol dehydrogenase (ODH, 1.1.1.1), peptidase A (valyl-leucine used as substrate; PEPA, 3.4.11), peptidase B (leucyl-glycyl-glycine used as substrate; PEPB, 3.4.11), peptidase C (leucyl-alanine used as substrate; PEPC, 3.4.11), peptidase D (phenylalanyl-proline used as substrate; PEPD, 3.4.13.9), peptidase F (leucyl-leucyl-leucine used as substrate; PEPF, 3.4.11), peptidase S (valyl-leucine, leucyl-alanine, or leucyl-glycyl-glycine used as substrate; PEPS, 3.4.11), phosphoglucomutase (PGM-1,-2,-3, 2.7.5.1), phosphoglucose isomerase (PGI, 5.3.1.9), 6-phosphogluconate dehydrogenase (6PGD, 1.1.1.44), sorbitol dehydrogenase (SDH, 1.1.1.14), and superoxide dismutase (SOD-1,-2, 1.15.1.1). Table 1 indicates which buffers were used for each protein system. All gels were subjected to 75-85 mA of current inside a refrigerated

cabinet for 6-8 h. Electromorphs were assumed to represent alleles and were assigned unique letters; the most anodal locus was designated as "locus 1" for enzymes in which the product of more than one gene locus (isozyme) was interpretable. Of the 41 loci surveyed (Table 2), 17 are absent from blood, and 5 are most easily detected using blood.

We sampled (Fig. 2) populations of Delmarva fox squirrels on the Eastern Shore of Maryland and Virginia (samples 1 and 2) as well as populations of fox squirrels from western Virginia (sample 3), western Maryland (sample 4), and Georgia (sample 5). Individuals from several localities in Moncrief's (1987) dissertation study were included to ensure proper assignment of allele designations for comparisons across studies (samples 6-10, Figs.2) . Loci included in Moncrief's (1987) dissertation study are noted in Table 2.

Because some proteins cannot be detected in blood, and some cannot be detected in tissue extracts, we treated individuals from sample 2 (Chincoteague National Wildlife Refuge, Virginia) as two separate samples in all calculations. This allowed direct comparisons between our results and those of Morgan and Quattro's (1986) study, which included only blood from Delmarva fox squirrels. Loci included in Morgan and Quattro's (1986) study are noted in Table 2.

Individuals from the 10 samples (Fig. 2) analyzed in this study were included on the same gel for each protein assayed. In addition, certain individuals served as internal controls; they

were analyzed for each enzyme on more than one gel, along with different subsets of individuals from each sample. These side-by-side comparisons were made to insure correct assessment of relative mobilities of electromorphs (i.e., to guarantee consistent "scoring" of alleles). This is the simplest method by which allozymic analyses can be "calibrated," and it allows proper comparison of results among populations and among gels.

The BIOSYS-1 program of Swofford and Selander (1981) was used to summarize and analyze statistically the electrophoretic results. As already noted, separate estimates were calculated for Chincoteague blood and Chincoteague tissue extracts, this is reflected in Tables 2 and 3. Percent polymorphism ($\underline{P}$) was calculated for each sample using loci for which the frequency of the most common allele was <95%. For each sample, mean heterozygosity ($\overline{\underline{H}}$) was calculated as the average proportion of heterozygous individuals at the loci examined (direct-count method), and the expected heterozygosity ($\overline{\underline{H}}_{exp}$, averaged over all loci and assuming Hardy-Weinberg equilibrium) was calculated for each sample using Nei's (1978) formula that corrects for small sample sizes. Genotypic proportions observed at each polymorphic locus were tested for conformation to the proportions expected under Hardy-Weinberg equilibrium. Chi-square tests using Levene's (1949) correction for small sample sizes were used to test for goodness-of-fit between observed and expected numbers of heterozygous individuals at each locus.

Estimates of intraspecific genetic variation from

electrophoretic studies must be interpreted in a comparative framework. Heterozygosity estimates and percentages of polymorphic loci cannot be subjected to quantitative analyses such as t-tests and ANOVA because the statistical distribution of their parameters is not known, and because these parameters may not conform to a normal distribution. Therefore, in order to characterize "typical" amounts of variation within and among populations of a particular species, well-designed electrophoretic studies measure variation within populations from several geographic regions of a species' distributional range.

Results

Delmarva fox squirrels (samples 1 and 2, Fig. 2, Tables 2 and 3) exhibited variation at 5 loci: MPI, NP, PEPB, PEPC, and PGM3. All populations of eastern fox squirrels (samples 1-5, Fig. 2, Tables 2 and 3) exhibited variation at MPI, NP, and PGM 3; two eastern samples (Western Maryland, sample 3, and Piedmont National Wildlife Refuge, Georgia, sample 5) also were variable at PGM2 (Tables 2 and 3). Delmarva fox squirrels (samples 1 and 2) possess an allele (MPI^f, Tables 2 and 3) not present in any other fox squirrel population analyzed to date, including the 14 populations of western fox squirrels analyzed by Moncrief (1987).

Mean heterozygosity ($\overline{H}$) in Delmarva fox squirrels (samples 1 and 2, Table 3) ranged from 0.022 to 0.038; $\overline{H}$ in other eastern

samples (sample 3-5, Fig. 2, Table 3) ranged from 0.019 to 0.040. Percentage of polymorphic loci ($\underline{P}$) ranged from 4.35% to 8.6% in Delmarva fox squirrels (samples 1 and 2, Table 3) and from 5.7% to 8.6% in the other eastern samples (samples 3-5, Table 3).

Chi-square tests revealed significant deviation from Hardy-Weinberg expectations at one locus each in the samples from Blackwater National Wildlife Refuge, Maryland and Piedmont National Wildlife Refuge, Georgia. The Blackwater sample (sample 1, Fig. 2) was deficient in heterozygotes at the PEPC locus (observed heterozygotes = 0, Table 2, and expected heterozygotes = 2, data not shown). Similarly, the Piedmont sample (sample 5, Fig. 2) was deficient in heterozygotes at the PGM2 locus (observed heterozygotes = 1, Table 2, and expected heterozygotes = 3, data not shown). There were no significant deviations from Hardy-Weinberg expectations in the sample of Delmarva fox squirrels from Chincoteague National Wildlife Refuge (data not shown).

Table 4 summarizes information about genetic variation at MPI, NP, PGM2, and PGM3 in the five western samples analyzed in Moncrief's (1987) dissertation study from which selected individuals were included in our analyses. For these five samples, our sample numbers and the abbreviations Moncrief (1987) used, respectively, are: 6, NH; 7, NE; 8, NV; 9, NB; 10, NX. Table 5 summarizes values for percent polymorphism ($\underline{P}$), mean heterozygosity ($\overline{\underline{H}}$), and expected heterozygosity ($\overline{\underline{H}}_{exp}$) reported by Moncrief (1987) in the 14 samples of western fox squirrels

analyzed in her study.

Discussion

Genetic variation in eastern fox squirrels (samples 1-5, Fig. 2, Tables 2 and 3) is comparable to variation reported in western fox squirrels by Moncrief (1987; Fig. 3, Tables 4 and 5). In that study, 14 samples from Arkansas, Tennessee, Texas, Louisiana, and Mississippi were analyzed at 35 loci (noted in Table 2).

Moncrief (1987) reported mean heterozygosity ($\overline{H}$) in western samples of fox squirrels (Fig. 3, Table 5) that ranged from 0.021 (sample NP) to 0.095 (sample NT). Percentage of polymorphic loci (P) in western samples (Fig. 3, Table 5) ranged from 5.7% (samples NA, NX, and NS) to 22.9% (sample NT). Our findings for $\overline{H}$ and P in eastern fox squirrels, including Delmarva fox squirrels, are within these ranges, and are within the ranges of values reported for other mammals by Nei and Graur (1984), Nevo (1978), and Powell (1975). The value of 4.35% P for the Chincoteague blood sample must be considered in light of the fact that MPI (which is the most variable locus in the other S. n. cinereus samples) cannot be detected in blood and thus cannot contribute to the polymorphism estimate for that sample.

Additional information regarding genetic information included estimates of heterozygote excess and deficiency. We

noted that the Blackwater sample (sample 1, Fig. 2, Table 2) is deficient in heterozygotes at PEPC and that the Piedmont sample (sample 5, Fig. 2, Table 2) is deficient in heterozygotes at PGM2. This result is probably not due to sampling error because each sample contained 40 individuals.

Moncrief (1987) noted the following excesses and deficiencies in heterozygotes in her study ("-" denotes deficiency, "+" denotes excess): NF (CK -, ME -); NK (PEPB +); NM (PGM2 -); NT (MDH1 -); NV (G6PD -, IDH2 -). There is no pattern, geographic or otherwise, in the deviations reported by Moncrief (1987) or those reported for eastern fox squirrels in this study. Additionally, the Chincoteague National Wildlife Refuge sample (sample 2), which underwent a recorded bottleneck (it was founded by 30 individuals, Dueser and Terwilliger, 1987), was not deficient in heterozygotes at its variable loci. The general absence of departure from Hardy-Weinberg expectations in S. niger is consistent with the findings of many allozymic studies of sexually outbreeding organisms (Smith et al., 1982).

The only other study of electrophoretic variation in S. n. cinereus, Morgan and Quattro's (1986) analysis of 57 Delmarva fox squirrels from Eastern Neck Island Wildlife Refuge and Dorchester County, Maryland, reported no electrophoretically detectable variation at 23 loci scored from blood. They (Morgan and Quattro, 1986) attributed the lack of variation in the populations they surveyed to genetic bottlenecking (Nei, et al., 1975) and the fact that populations of S. n. cinereus on Eastern

Neck Island were introduced from Blackwater Refuge in 1966. They were unable to assay their samples for MPI because MPI is not present in red blood cells. MPI was one of four variable loci in the S. n. cinereus populations we surveyed (including Chincoteague National Wildlife Refuge, which was represented by only 6 tissue extracts in this study).

In contrast to Morgan and Quattro's findings, our results indicate that S. n. cinereus is not devoid of detectable genetic variation: we found levels of variation in Delmarva fox squirrels that is comparable to that present in other eastern populations (Table 2 and 3). Moreover, the Chincoteague National Wildlife Refuge population, which was established by no more than 30 individuals (Dueser and Terwilliger, 1987), is not totally homozygous, and it did not exhibit significant deviations from Hardy-Weinberg expectations for those loci at which there is variation. This finding is encouraging, but does not alter the fact that the Chincoteague National Wildlife Refuge is in immediate danger of becoming extinct, due to it's precarious location on a barrier island (Dueser and Terwilliger, 1987).

Our analyses detected an allele (MPI^f) in S. n. cinereus (samples 1 and 2, Fig. 2) that is not present in any other population of S. niger examined to date, including Moncrief's (1987) 14 western populations. Analyses of other populations of eastern fox squirrels, especially those from within and adjacent to the historic range of S. n cinereus (Delaware, Pennsylvania, New Jersey), are necessary before this allele can be considered

unique and characteristic of Delmarva fox squirrels. These analyses may also identify those populations that are most closely related to Delmarva fox squirrels. Whether of not this allele is informative as a genetic marker, this electrophoretically detectable attribute represents genetic variation that is more easily quantified than variation in coat coloration, which certainly exists in S. n. cinereus (pers. obs.). Additional techniques (e.g., restriction site analysis and sequencing of mitochondrial and nuclear DNA) should reveal additional quantifiable genetic variation within and among S. n. cinereus populations.

In conclusion, our analyses indicate that overall levels of genetic variation in Delmarva fox squirrels (as measured by horizontal starch-gel protein electrophoresis) are comparable to levels of genetic variation in other fox squirrels examined to date. Our study also revealed an electrophoretically detectable genetic attribute (the MPI^f allele) that may be unique to S. n. cinereus populations. Further electrophoretic analysis of Delmarva and other eastern fox squirrels is necessary to determine whether this attribute is a genetic marker for identification of Delmarva fox squirrels. Additional techniques, including restriction site analysis and sequencing of mitochondrial and nuclear DNA, should further elucidate genetic variation within and among S. n. cinereus populations and their closest relatives. Although our findings are encouraging, in that there is genetic variation in S. n. cinereus (contrary to

Morgan and Quattro's, 1986 report), the long-term security and sustainability of Delmarva fox squirrel populations remains uncertain.

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Literature Cited

Dueser, R. D. and Terwilliger, K. 1987. Status of the Delmarva fox squirrel (Sciurus niger cinereus) in Virginia. Virginia Journal of Science, 38: 380-388.

Hall, E. R. 1981. The mammals of North America. Second ed. John Wiley and Sons, New York, 1:1-606 + 90.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam, looseleaf.

Levene, H. 1949. On a matching problem arising in genetics. Annals of Mathematics and Statistics, 20:91-94.

Moncrief, N. D. 1987. Geographic variation in morphology and allozymes within tree squirrels, Sciurus niger, and S. carolinensis of the lower Mississippi River valley. Unpubl. Ph.D. Dissertation, Louisiana State University, Baton Rouge. 154 pp.

Morgan, R. P., II and J. M. Quattro. 1986. Hybridization in Delmarva fox squirrels. Final Report. U.S. Fish and Wildlife Service.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89:583-590.

Murphy, R. W., J. W. Sites, Jr., D. G. Buth, and C. H. Haufler. 1990. Pp. 45-126 In Molecular Systematics (D. M. Hillis and C. Moritz, eds.). Sinauer Associates, Inc., Sunderland, MA. 588 pp.

Nei, M., and D. Graur. 1984. Extent of protein polymorphism and the neutral mutation theory. Evolutionary Biology, 17:73-118.

Nei, M., T. Maruyama, and R. Chakrabozty. 1975. The bottleneck effect and genetic variability in populations. Evolution, 29:1-10.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. Theoretical Population Biology, 13:121-177.

Powell, J. R. 1975. Protein variation in natural populations of animals. Evolutionary Biology, 8:79-119.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Studies in Genetics IV, The University of Texas Publication, 7103:49-90.

Smith, M. W., C. F. Aquadro, M. H. Smith, R. K. Chesser, and W. J. Etges. 1981. A bibliography of electrophoretic studies of biochemical variation in natural populations of vertebrates. Institute of Ecology, University of Georgia, Athens, 158 pp.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity, 72:281-283.

Table 1.-- Buffers used and loci analyzed. TC7 = tris citrate pH 7.0; TC8 = tris citrate pH 8.0; P = Poulik. Abbreviations for loci are given in text.

	Buffer		
Locus	TC7	TC8	P
SOD2	X		
ACP	X	X	
HK		X	
AK		X	X
CK		X	X
ACN1	X	X	
SOD1	X		X
FUM	X		X
G6PD		X	
GLUD	X	X	
GOT1	X	X	
GOT2	X	X	
GDA	X		
G3PD		X	X
IDH1	X	X	
IDH2	X	X	
SDH	X		
LDH1	X		
LDH2	X		
MDH1	X	X	
MDH2	X	X	
ME	X	X	X
MPI	X		
NP	X		
ODH1	X		
Hb	X	X	
PEPF		X	
EAP	X		
GR	X		
DIA	X		
PEPD		X	X
PEPA		X	X
PEPB		X	X
PEPS		X	X
PEPC		X	X
6P6D		X	
PGI		X	X
PGM1	X		
PGM2	X		
PGM3	X		
ADH	X		

Table 2. -- Genotypes at 41 loci for 10 samples of fox squirrels (Sciurus niger).

		(Sample)									
		(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
		Blkwtr	Chincoteague VA		Western	Western	Piedmont	Holmes	EastBR	Vernon	Bossier	Atascosa
		MD	Blood	Tissues	VA	MD	GA	MS	LA	LA	LA	TX
Locus	N	34	40	6	40	18	40	4	1	1	1	1
SOD2		34 mm	?[1]	6 mm	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]
ACP[2,3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
HK[2]		?[1]	36 mm	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]	?[1]
AK[3]		34 mm	40 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
CK[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
ACN1[3]		32 mm	---[4]	2 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
SOD1[2,3]		?[1]	36 mm	?[1]	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
FUM[3]		34 mm	---[4]	5 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
G6PD[2,3]		33 mm	40 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
GLUD[3]		33 mm	---[4]	6 mm	33 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
GOT1[3]		34 mm	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
GOT2[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
GDA[3]		29 mm	---[4]	2 mm	34 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
G3PD[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
IDH1[2,3]		34 mm	36 mm	6 mm	37 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
IDH2[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
SDH[2,3]		34 mm	40 mm	6 mm	35 mm	18 mm	38 mm	4 mm	1 mm	1 mm	1 mm	1 mm
LDH1[2,3]		34 mm	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm

Table 2. -- continued.

Locus	N	(Sample) (1) Blkwtr MD 34	(2) Chincoteague VA Blood 40	(2) Chincoteague VA Tissues 6	(3) Western VA 40	(4) Western MD 18	(5) Piedmont GA 40	(6) Holmes MS 4	(7) EastBR LA 1	(8) Vernon LA 1	(9) Bossier LA 1	(10) Atascosa TX 1
LDH2[3]		34 mm	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
MDH1[2,3]		34 mm	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
MDH2[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
ME[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
MPI[3]		12 mm 16 ms 3 ss 1 ff 1 fs 1 fm	---[4]	2 mm 1 ms 1 ss 1 fs 1 fm	15 mm 18 ms 7 ss	5 mm 10 ms 3 ss	2 mm 10 ms 28 ss	1 mm 2 ms 1 ss	1 mm	1 mm	1 mm	1 mm
NP[2,3]		4 ss 21 fs 9 ff	12 ss 20 fs 8 ff	1 ss 4 fs 1 ff	10 ss 16 fs 14 ff	4 ss 9 fs 5 ff	23 ss 14 fs 3 ff	4 fs	1 ss	1 fs	1 fs	1 ff
ODH1[3]		?[1]	---[4]	?[1]	33 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
Hb[2]		---[5]	40 mm	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]
PEPF		---[5]	39 mm	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]
EAP		---[5]	39 mm	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]
GR		---[5]	36 mm	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]
DIA[2]		---[5]	36 mm	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]	---[5]
PEPD[3]		34 mm	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
PEPA[3]		34 mm	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm

Table 2. -- continued.

		(Sample)									
		(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
		Blkwtr	Chincoteague VA		Western	Western	Piedmont	Holmes	EastBR	Vernon	Bossier	Atascosa
		MD	Blood	Tissues	VA	MD	GA	MS	LA	LA	LA	TX
Locus	N	34	40	6	40	18	40	4	1	1	1	1
PEPB[3]		32 mm 2 ms	39 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
PEPS[3]		34 mm	---[4]	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
PEPC[3]		33 mm 1 ff	39 mm	6 mm	?[1]	18 mm	?[1]	4 mm	1 mm	1 mm	1 mm	1 mm
6PGD[2,3]		33 mm	40 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
PGI[2,3]		34 mm	36 mm	6 mm	40 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
PGM1		34 mm	---[4]	6 mm	38 mm	18 mm	40 mm	4 mm	1 mm	1 mm	1 mm	1 mm
PGM2[2,3]		34 mm	40 mm	6 mm	?[1]	12 mm 5 ms 1 ss	36 mm 1 ms 1 ss	3 mm 1 ms	1 mm	1 ms	1 mm	1 ss
PGM3[3]		31 mm 3 fm	---[4]	5 mm 1 fm	29 mm 10 fm 1 ff	17 mm 1 fm	39 mm 1 fm	3 mm 1 fm	1 mm	1 mm	1 mm	1 mm
ADH		?[1]	---[4]	?[1]	22 mm	16 mm	?[1]	4 mm	1 mm	1 mm	1 mm	1 mm

[1] data not available.

[2] indicates proteins included in Morgan and Quattro's (1986) study; they also analyzed adenosine deaminase (3.5.4.4), catalase (1.11.1.6), esterase (3.1.1.1), xanthine dehydrogenase (1.2.1.37), and serum proteins.

[3] indicates protein included in Moncrief's (1987) study. She also analyzed adenosine deaminase (3.5.4.4), creatine kinase-1, and aconitase-1.

[4] not present in blood.

[5] not present in heart, liver, kidney, skeletal muscle.

Table 3.-- Allele frequencies at loci variable in more than one sample of eastern fox squirrels (Sciurus niger), mean heterozygosity (H), number of expected heterozygotes (H_{exp}; Nei, 1978), and percent polymorphism (P). Abbreviations for loci are given in the text. Geographic location of samples is indicated in Fig. 2.

	(Sample)					
	(1) Blackwater MD	(2) Chincoteague VA blood	(2) Chincoteague VA tissues	(3) Western VA	(4) Western MD	(5) Piedmont GA
Locus	N 34	40	6	40	18	40
MPI	0.60 m 0.34 s 0.06 f	---[1]	0.50 m 0.33 s 0.17 f	0.60 m 0.40 s	0.56 m 0.44 s	0.18 m 0.82 s
NP	0.43 s 0.57 f	0.55 s 0.45 f	0.50 s 0.50 f	0.45 s 0.55 f	0.48 s 0.52 f	0.75 s 0.25 f
PGM2	1.00 m	1.00 m	1.00 m	?	0.81 m 0.19 s	0.96 m 0.04 s
PGM3	0.96 m 0.04 f	---[1]	0.92 m 0.08 f	0.85 m 0.15 f	0.97 m 0.03 f	0.99 m 0.01 f
$\bar{H}$	0.037	0.022	0.038	0.031	0.040	0.019
$\bar{H}_{exp}$	0.035	0.022	0.039	0.036	0.040	0.022
P	5.7	4.35	8.6	8.6	8.6	5.7

[1]not present in blood

Table 4.-- Allele fequencies at selected loci that are variable in five samples of western fox squirrels (Sciurus niger), mean heterozygosity (H), number of expected heterozygotes (H_{exp}; Nei, 1978), and percent polymorphism (P). Data are from Moncrief (1987). Abbreviations for loci are given in the text. Geographic location of samples is indicated in Figs. 2 and 3.

		(Sample)				
		(6) Holmes MS	(7) East BR LA	(8) Vernon LA	(9) Bossier LA	(10) Atascosa TX
Locus	N	13	10	7	5	1
MPI		0.69 m	0.95 m	1.00 m	1.00 m	1.00 m
		0.31 s	0.05 s			
NP		0.31 s	0.85 s	0.43 s	0.80 s	
		0.69 f	0.15 f	0.57 f	0.20 f	1.00 f
PGM2		0.89 m	1.00 m	0.79 m	1.00 m	
		0.11 s		0.21 s		1.00 s
PGM3		0.96 m	1.00 m	1.00 m	1.00 m	1.00 m
		0.04 f				
H		0.047	0.041	0.057	0.036	0.057
H_{exp}		0.047	0.035	0.062	0.033	0.057
P		11.4	17.1	17.1	11.4	5.7

Table 5.-- Values for mean heterozygosity ($\overline{H}$), number of expected heterozygotes ($\overline{H}_{exp}$; Nei, 1978), and percent polymorphism (P) in 14 samples of western fox squirrels (Sciurus niger). Data are from Moncrief (1987). See Fig. 3 for sample abbreviations and geographic location of samples.

Sample	N	$\overline{H}$	$\overline{H}_{exp}$	P
NA	12	0.026	0.033	5.7
NB	5	0.036	0.033	11.4
NE	10	0.041	0.035	17.1
NF	6	0.048	0.065	17.1
NH	13	0.047	0.047	11.4
NJ	11	0.034	0.040	8.6
NK	5	0.073	0.057	11.4
NM	7	0.024	0.040	11.4
NP	4	0.021	0.030	8.6
NT	3	0.095	0.122	22.9
NV	7	0.057	0.062	17.1
NX	1	0.057	0.057	5.7
NW	3	0.057	0.057	11.4
NS	5	0.034	0.029	5.7

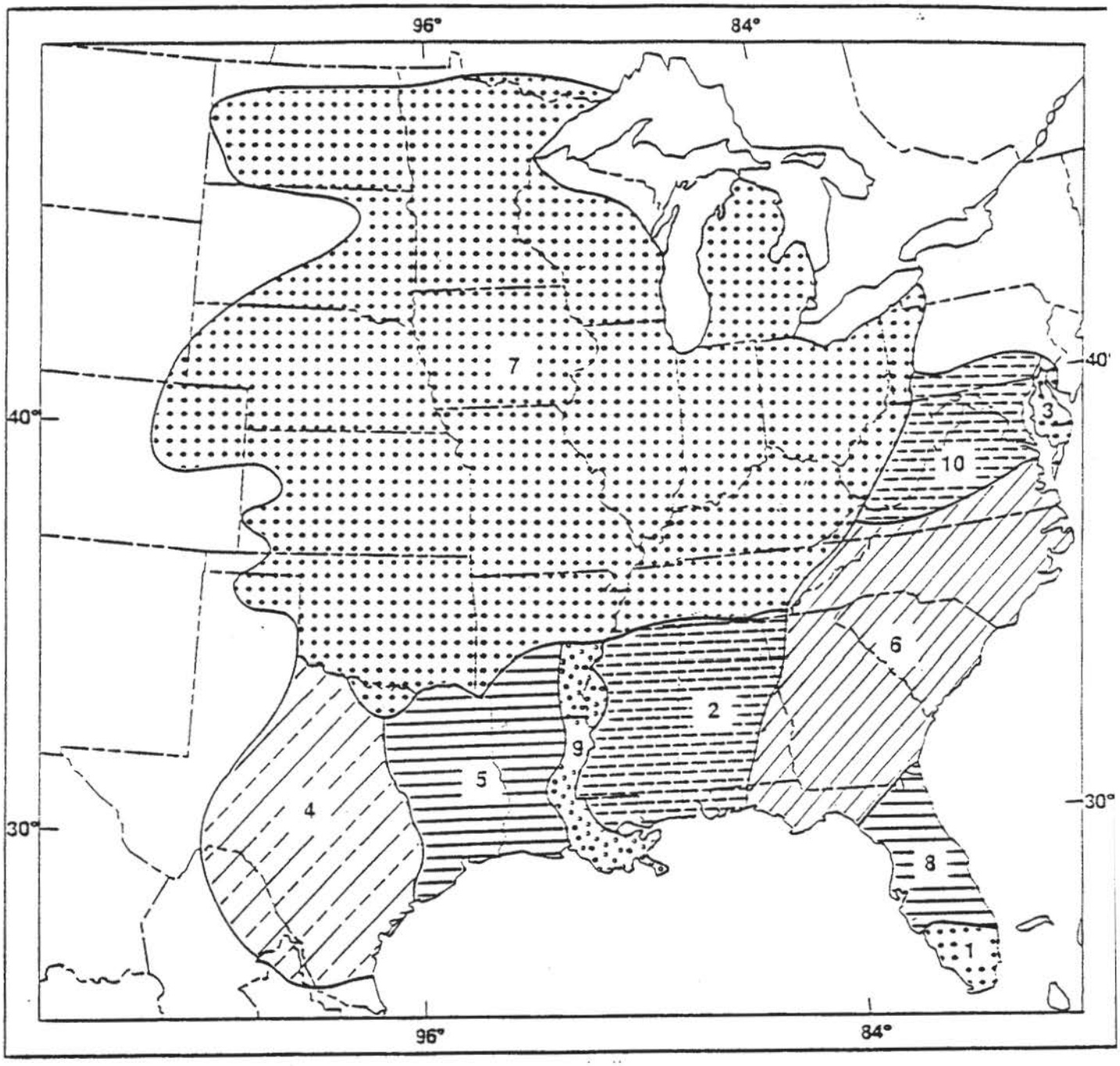

Fig. 1.– Geographic range and subspecies distribution of the fox squirrel, Sciurus niger: 1 = S. n. avicennia; 2 = S. n. bachmani; 3 = S. n. cinereus; 4 = S. n. limitis; 5 = S. n. ludovicianus; 6 = S. n. niger; 7 = S. n. rufiventer; 8 = S. n. shermani; 9 = S. n. subauratus; 10 = S. n. vulpinus.

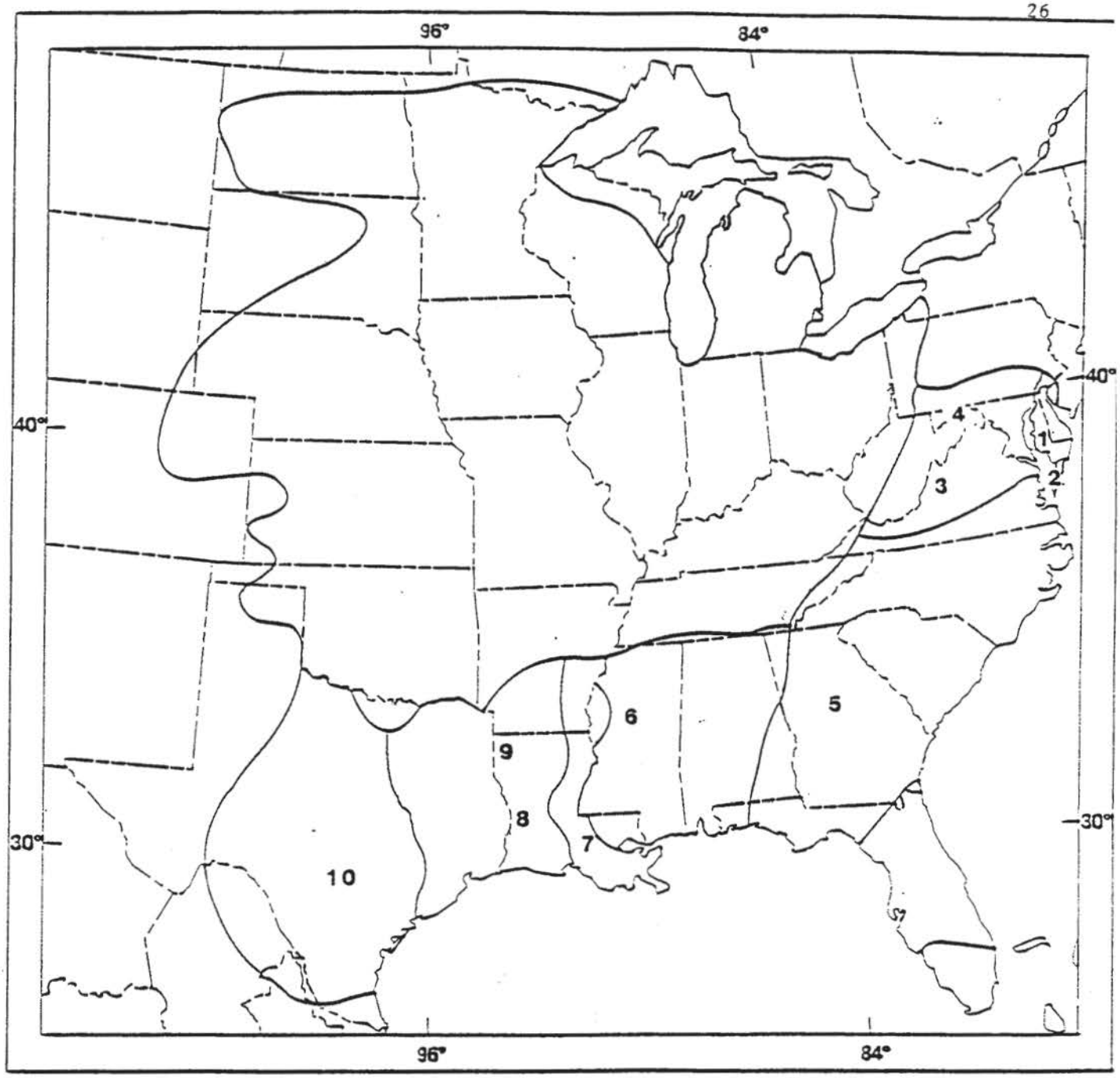

Fig. 2.-- Location of Sciurus niger samples included in this study: 1= Maryland: Dorchester Co., Blackwater National Wildlife Refuge; 2 = Virginia: Accomack Co., Chincoteague National Wildlife Refuge; 3 = Virginia: Alleghany, Augusta, Botetourt, Craig, Giles, Highland, Rockbridge, Rockingham, Russell, Shenandoah, Smyth, Washington, and Wythe Cos.; 4 = Maryland: Allegany Co.; 5 = Georgia: Jasper and Jones Cos., Piedmont National Wildlife Refuge; 6 = Mississippi: Holmes Co.; 7 = Louisiana: East Baton Rouge Par.; 8 = Louisiana: Vernon Par.; 9 = Louisiana: Bossier Par.; 10 = TX: Atascosa Co.

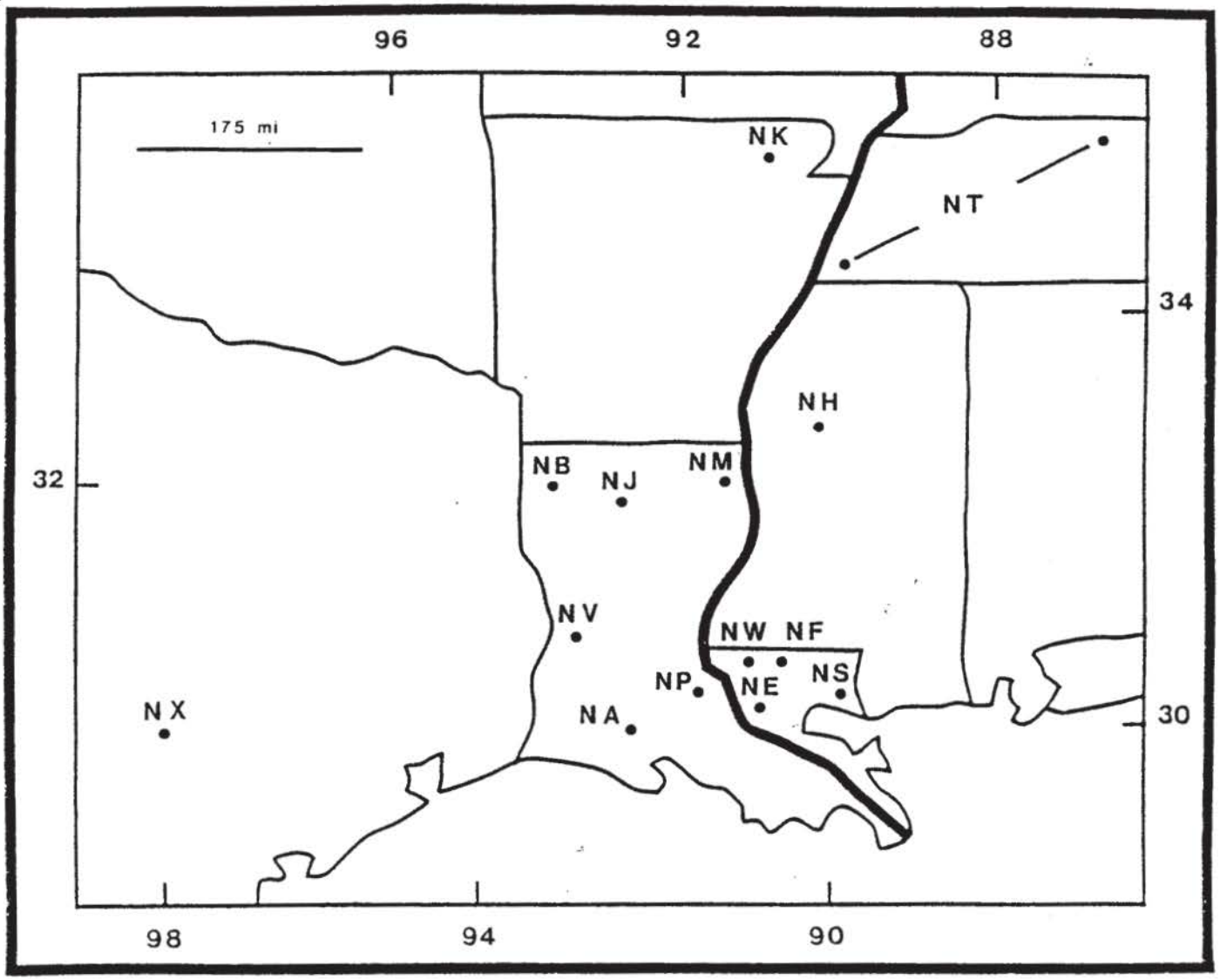

Fig 3.-- Samples included in Moncrief's (1987) study of western fox squirrels (Sciurus niger): **NA** = Louisiana: Acadia Par.; **NB** = Louisiana: Bossier Par.; **NJ** = Louisiana: Jackson, Bienville, and Winn Pars.; **NV** = Louisiana: Vernon, and Grant Pars.; **NE** = Louisiana: Ascension, East Baton Rouge, and Iberville Pars.; **NM** = Louisiana: Madison Par.; **NP** = Louisiana: Pointe Coupee Par.; **NF** = Louisiana: East Feliciana Par.; **NH** = Mississippi: Holmes Co.; **NS** = Louisiana: St. Tammany Par.; **NW** = Louisiana: West Feliciana Par.; **NX** = Texas: Atascosa Co.; **NK** = Arkansas: Greene Co.; **NT** = Tennessee: Haywood, McNairy, and Trousdale Cos.