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Genetic variation in natural and translocated populations of the endangered Delmarva fox squirrel (*Sciurus niger cinereus*)

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Abstract

The Delmarva fox squirrel, *Sciurus niger cinereus*, is a federally listed endangered subspecies whose range has been reduced by 90%. In an attempt to increase both population size and range, translocation sites were established beginning in the 1960's by moving squirrels from the natural range to sites outside the current range. Although translocations have served as the primary component of the DFS recovery program, there has been very little post-release examination of the genetics of the translocation sites. In this study, we developed ten microsatellite loci, screened the three polymorphic loci, and sequenced a 330 bp fragment of the mitochondrial control region in order to assess levels of genetic variation in natural and translocated regions of Delmarva fox squirrels and to compare them to Southeastern fox squirrels (*S. n. niger*). Although we found low levels of microsatellite polymorphism, there were no differences in heterozygosity between natural and translocated regions, or between Delmarva and Southeastern fox squirrels. We found high levels of polymorphism in the mitochondrial control region. Our patterns of haplotype diversity suggest incomplete lineage sorting of the two subspecies. In general, our data suggest that the current levels of genetic variation in the translocated sites are representative of those found in the natural population, and we encourage the continued use of translocations as a major component of Delmarva fox squirrel recovery.

Introduction

The Delmarva fox squirrel, *Sciurus niger cinereus*, has been listed under the Endangered Species Act of the Unites States of America since 1967 (U.S. Fish and Wildlife Service 1993). Historically, Delmarva fox squirrels (DFS) ranged throughout the Delmarva Peninsula, extending into southeastern Pennsylvania and southern New Jersey (Lustig and Flyger 1975). Today, DFS populations remain in only 10% of their historic range and are primarily found in portions of four counties on Maryland's Eastern Shore (Figure 1). The human population on the Delmarva Peninsula is growing rapidly and habitat loss due to a combination of timber harvest, short-rotation pine forestry, and conversion of forests for agricultural and structural development has been considered the primary cause of DFS' decline (Maryland Forest, Park and Wildlife Service 1989; Taylor 1973). Comparisons of occupied to unoccupied habitat suggest that DFS prefer mature forests that have high percentages of large trees, relatively low percentages of shrub groundcover, and low understory densities (Dueser et al. 1988). The combination of human population growth and DFS' preference for mature forests suggests that management will play an important role in the future of DFS.

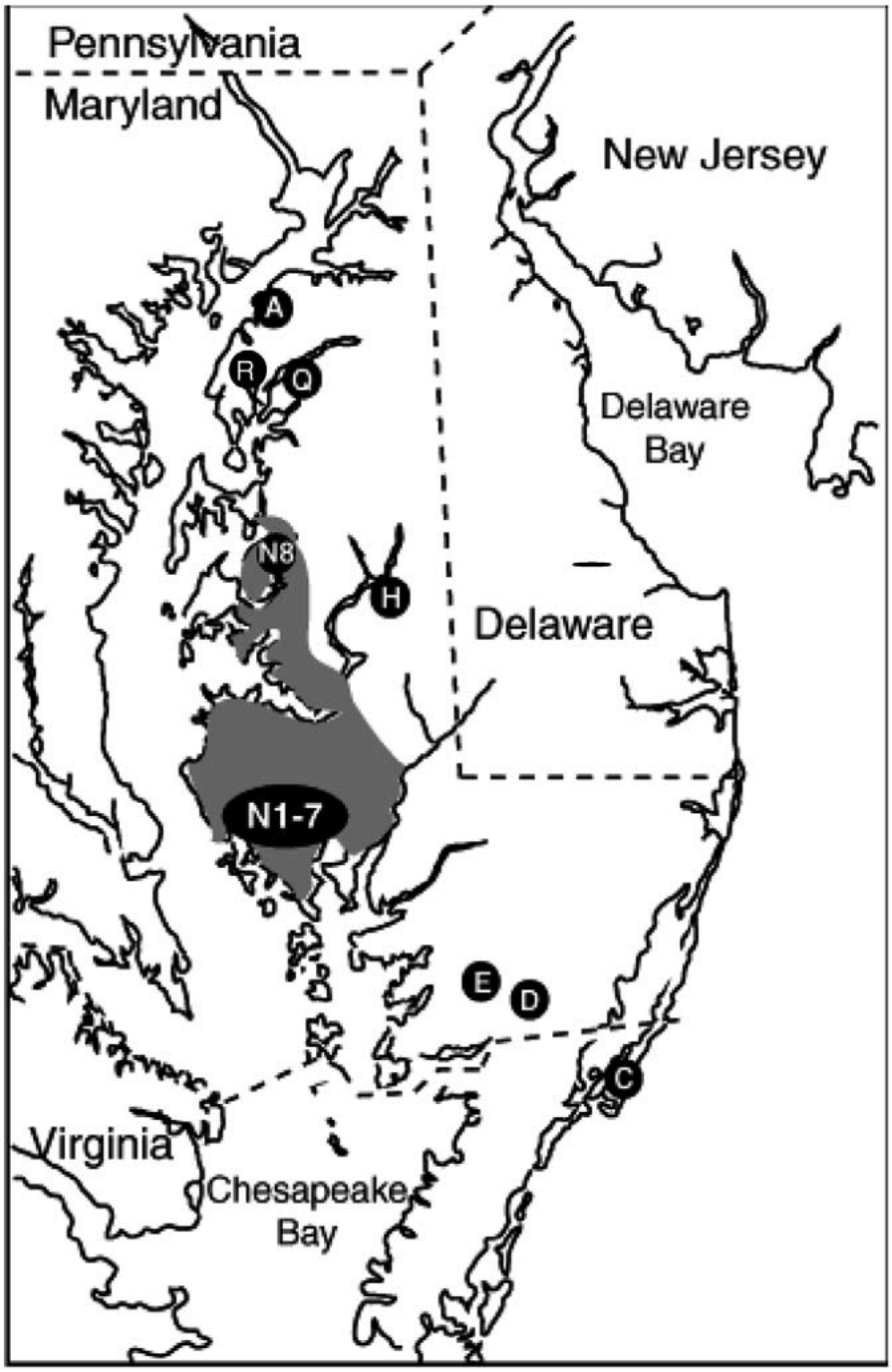

Figure 1. Current distribution (shaded area) and sampling locations of DFS. Sampling localities or translocated sites are as follows: Andelot Farm (A), Quaker Neck (Q), Eby Farm (E), Dryden Farm (D), Harmony (H), Remington Farms (R) and Chincoteague National Wildlife Refuge (C). The natural sampling localities Jarrett East, Jarrett West, Kuehnle, White Marsh, Hayes Farm, and TCF 1-3, are all within the area labeled N1-7 and are no more than 10 km away from each other and connected by contiguous suitable habitat. The natural sampling locations, Homeport and Chino Farms are within N8.

Three major conservation efforts have been undertaken on behalf of DFS. First, a Recovery Team was assembled, and in 1979 an initial Recovery Plan was approved. Second, in an attempt to reverse the decline of the DFS, 17 translocation sites were established by moving squirrels from the natural range to sites outside the current range, but within the former range. Third, in 1990, seven benchmark sites were established for the long-term monitoring of DFS.

Translocations have served as the primary component of the DFS recovery program. From 1968 to 1989, a total of 264 squirrels were moved to 11 sites in Maryland (Therres and Willey, in press), two in Delaware (Reynolds 1988), two in Virginia (Dueser and Terwilliger 1987), and one in Pennsylvania (Dunn 1989). In most cases, source squirrels were taken from multiple locations. Initially, the sites received an average of 16.5 squirrels (U.S. Fish and Wildlife Service 1993). After 1990, six translocations received supplemental squirrels in an attempt to increase the average number of squirrels to 24 squirrels/site and an additional site, Andelot Farm, was established with 42 squirrels (Therres and Willey, in press). Four of the translocation sites in this study were supplemented with squirrels. Eby was supplemented with 17 squirrels in 1993, Remington Farms (previously referred to as DeBlasio Tract in the DFS Recovery Plan; U.S. Fish and Wildlife Service 1993) with 25 squirrels in 1994, Dryden with 19 squirrels in 1999, and Quaker Neck with 18 squirrels in 2000. All translocated squirrels are ear tagged with individual identification numbers. Although these sites were supplemented, all of our translocation samples came from unmarked squirrels and thus represent recruitment.

To determine whether translocations are a viable option for continued protection of DFS, it is imperative to evaluate previous translocations, the factors that contributed to their success or failure, and whether they resulted in populations with potential for long-term survival. For this reason, the DFS Recovery Plan (U.S. Fish and Wildlife Service 1993) specifically establishes that the determination of the current status of DFS translocations be considered a research priority (Recovery Task 1.23). Periodic surveys of the 11 Maryland translocation sites have determined that reproduction has occurred in nine of them, but they have not been genetically analyzed. In addition, the catch-per-unit-efforts reported at the translocated sites suggest an increase in numbers to levels on par with naturally occurring locations (Therres and Willey, in press).

As detailed above, each translocation site was established with a small number of individuals. Such population size reductions, or bottlenecks, may result in a loss of genetic variation (Wright 1931; Nei et al. 1975; Chakraborty and Nei 1976). Reduced genetic variation is associated with an inability to evolve in response to environmental changes (see Frankham et al. 2002 and references therein) and with increased susceptibility to diseases and parasites (Falk and Holsinger 1991; Frankham 1995). Clearly, sufficient genetic variation is essential for the long-term viability of the translocation sites.

The purpose of this study was twofold: (1) to examine genetic variation in the natural range of DFS and (2) to compare genetic variation between natural and translocated locations of DFS. The results of our genetic analysis are used to recommend management strategies for the translocation sites.

Methods

Sample collection and DNA extraction

DFS hair samples were collected from 90 individuals from nine translocation sites and from 110 individuals from 10 locations within the remaining natural range from October of 1998 to October 2000 (Figure 1 and Table 1). All but two samples from the natural range came from the trapping efforts of USGS Patuxent Wildlife Research Center as part of a larger ecological study. Two of the natural locations, Chino Farms and Hayes Farm, had sample sizes of one and therefore were excluded from genetic analyses, but were included in the sample and haplotype tables for reference (Tables 1 and 5). Samples from translocation sites came from a combination of nest box checks and trapping efforts at benchmark sites, road kill specimens, and trapping efforts conducted specifically for this project (USFWS endangered species permit #697823). In all cases, trapping efforts followed the protocol outlined in the Delmarva fox squirrel recovery plan. In addition, hair and blood samples of 25 SFS from six locations in South Carolina, and hair samples from three road killed *S. n. vulpinus* (WFS) from Virginia were also analyzed (Table 1).

Eight of our SFS samples came from Spring Island, South Carolina, an isolated island, and a single sample was obtained from a fox squirrel from the University

Table 1. Sampling locations and sizes for natural (Nat) and translocated (Trans) populations of *Sciurus niger*. Locality codes correspond to localities in Figure 1

Subspecies	Acroynym	Locality (code)	County	State	Natural or translocated	Number sampled	Microsatellites (n)	MtDNA (n)
S. n. cinereus	DFS	Andelot (A)	Kent	MD	Trans	15	14	13
S. n. cinereus	DFS	CNWR (C)	Accomack	VA	Trans	34	33	21
S. n. cinereus	DFS	Eby Farm (E)	Somerset	MD	Trans	18	18	9
S. n. cinereus	DFS	Quaker Neck (Q)	Kent	MD	Trans	4	4	1
S. n. cinereus	DFS	Dryden (D)	Somerset	MD	Trans	4	3	3
S. n. cinereus	DFS	Harmony (N8)	Caroline	MD	Trans	13	13	12
S. n. cinereus	DFS	Remington Farms (R)	Kent	MD	Trans	5	3	3
S. n. cinereus	DFS	Jarrett West (N1-7)	Dorchester	MD	Nat	28	27	20
S. n. cinereus	DFS	Kuehnle (N1-7)	Dorchester	MD	Nat	28	28	21
S. n. cinereus	DFS	Jarrett East (N1-7)	Dorchester	MD	Nat	13	13	12
S. n. cinereus	DFS	White Marsh (N1-7)	Dorchester	MD	Nat	13	13	11
S. n. cinereus	DFS	TCF 1 (N1-7)	Dorchester	MD	Nat	11	11	11
S. n. cinereus	DFS	TCF 2 (N1-7)	Dorchester	MD	Nat	8	8	8
S. n. cinereus	DFS	TCF 3 (N1-7)	Dorchester	MD	Nat	8	8	8
S. n. cinereus	DFS	Homeport (N1-7)	Queen Anne's	MD	Nat	4	2	2
S. n. cinereus	DFS	Hayes Farm (N1-7)	Dorchester	MD	Nat	1	1	1
S. n. cinereus	DFS	Chino Farms (N8)	Queen Anne's	MD	Nat	1	1	1
S. n. niger	SFS	Spring Island	Beaufort	SC	Nat	9	7	8
S. n. niger	SFS	Allendale	Allendale	SC	Nat	8	8	4
S. n. niger	SFS	Garnett	Hampton	SC	Nat	3	3	3
S. n. niger	SFS	Twickenham	Beaufort	SC	Nat	2	2	2
S. n. niger	SFS	Yemassee	Beaufort	SC	Nat	3	3	2
S. n. niger	SFS	Unknown		SC	Nat	3	1	3
S. n. vulpinus	WFS	Ft. Defiance	Augusta	VA	Nat	2	0	2
S. n. vulpinus	WFS	Rte 257/Rte 613	Rockingham	VA	Nat	1	0	1
S. n . rufiventer	WFS	Westwood	Los Angeles	CA	Trans	1	0	1

of California at Los Angeles campus. The squirrels at UCLA represent one of the many introductions to California (Ingles 1965), and the source location is unknown but is most likely *S. niger rufiventer*. Genomic DNA was isolated from all samples using standard protocols included in the QIAGEN Dneasy® Tissue Kit.

Microsatellite isolation and amplification

Microsatellites were isolated from DFS and SFS genomic libraries enriched for GT and CT repeats following previously described methods (Hamilton et al. 1999). Forward and reverse primers were developed for 18 clones, which contained at least 10 repeats and 40 bp of 5′ and 3′ flanking sequence. Ten of these loci were successfully optimized for PCR (Table 2) of which five were isolated in DFS and five in SFS. PCR was carried out in a 25 μl cocktail containing between 50 and 250 ng of genomic DNA, 10 mM dNTP's with fluorescent dNTP's incorporated, 1 μM each of a forward and reverse primer, 2 mM of MgCl2, 10 mM Tris-HCl, pH 8.3, 50 mM KCl and 1.25 units of *AmpliTaq*® DNA Polymerase. The cycling conditions consisted of 5 min at 96 °C followed by 30 cycles of: 1 min at 94 °C, 1 min at appropriate annealing temperature, and 1 min 30 s at 72 °C. Amplifications were performed in an MJ Research, Inc. PTC 100® Programmable Thermal Cycler. PCR reactions were cleaned by centrifugation through Sephadex columns and subsequently 6 μl were dried down in a speed-vacuum. Samples were resuspended in formamide loading dye containing 0.2 μl of the internal size standard Rox (ABI systems, Foster City, CA, USA) and alleles were separated in a polyacrylamide gel using an ABI 373 or ABI

Table 2. Summary of ten microsatellite loci developed for *Sciurus niger cinereus* (DFS) and *S. n. niger* (SFS)

Locus	Forward primer: 5′ to 3′	Reverse primer: 5′ to 3′	Repeat type	Annealing temp. (°C)	Number of alleles	Allele size range
DFS 3	CCACCCTGATCCTAACCAAC	TTAGTRGCGTGGTGACTGGC	$(CA)_{21}$	62	1	239
DFS 27	TCCAATGATGCTCTTTGGCT	TGTTTAATTCCAGCACCTCC	$(CA)_{15}$	56	7	145–159
DFS 33	CATCACGCATGTAATTTCAG	CATCTATGGAGGAGTAAGGG	$(GT)_2GCTG(GT)_{11}$	60	1	156
DFS 38	GGCATCTTTCAAGATCCACT	GGGCATACAGTAATTGTAGCC	$(CA)_{16}$	62	1	255
DFS 45	TCAATGGTAGGGAGCATGCC	GGTTTCCTGTCTCTGCAGCC	$(CA)_{12}GA(CA)_5$	48	1	148
SFS 7	GAGGTGGAACTCATTTGAGG	GCTGGTCACCAGGCACAGGC	$(TC)_{15}$	62	1	146
SFS 22	CAAATGACTCTATGCTGTCC	TGCTCCTCTTAGGTTCCCAC	$(CA)_4CT(CA)_9$	62	1	158
SFS 36	AGCAGAGGATGCTACAAGTGC	AAAGCCCCATAACAGGTTCC	$(CA)_{13}$	58	12	186–212
SFS 43	GCCTTCCTGGATCATGAGGC	CTGGTGATGTAACTCAGTGGG	$(CT)_{22}$	62	10	148–178
SFS 45	GCTCAGCATGTGCAAGACCC	GGGAATGCTAAAACTAGTGG	$(CA)_{15}T(AC)_3$	58	1	171

377 automated sequencer and analyzed with Genescan (Applied Biosystems 1994). Products from several individuals were amplified multiple times to verify that genotyping remained consistent. In addition, amplicons from several individuals were run on all gels to confirm that electrophoresis remained constant.

Microsatellite analysis

All microsatellite analyses were computed using FSTAT 2.9.3 (Goudet 2001). Deviations from Hardy-Weinberg equilibrium were examined based on 1000 permutations of a randomization test. Due to unequal sample sizes we also estimated allelic richness (Rs) per locus and sampling location. FSTAT employs an adaptation of the rarefaction index (El Mousadik and Petit 1996) to estimate the expected number of alleles in a sub-sample of genes. The number of genes sub-sampled depends upon the size of the smallest sample. Differences in Rs and observed proportions of heterozygotes (Ho, Nei 1987) among DFS translocations, DFS natural locations, and SFS locations were examined using randomization tests with 1000 permutations. For Rs estimates and randomization tests we excluded samples with fewer than five genotyped individuals and thus our smallest sample contained seven individuals. Our Rs estimate for SFS is based on a pooled sample. Gene flow among sampling locations was approximated with Weir and Cockheram (1984) estimators of F_{ST} values.

Mitochondrial DNA sequencing

A ∼600 bp fragment of the mtDNA control region was initially amplified from several DFS with universal primers L15926 and H16340 (Kocher et al. 1989). Internal primers DFSloopf (5′CGCAATACTCGACCAATTCC-3′) and DFSloopr (5′TGATGATTTCACGGAGGTAGG-3′) were subsequently developed to amplify a shorter (∼410 bp) fragment of the control region and to increase our amplification success rate with hair samples. Amplification conditions were as above except that no fluorescent dNTP's were incorporated and the annealing temperature was 55 °C. All PCR reactions were cleaned with QIAquick™ PCR purification columns (Qiagen, Valencia, CA, USA). Cycle sequencing reactions were conducted on the cleaned products using a one-quarter reaction of BigDye™ Terminator Cycle Sequencing Ready Reaction mix (Applied Biosystems, Foster City, CA). Sequences were determined in a model 377 automated sequencer (PE Biosystems, Foster City, CA). We successfully sequenced a 330 bp fragment for 157 DFS, 23 SFS, 3 WFS and one presumed *S. n. rufiventer* (GenBank accession numbers AF533252–AF533274).

Sequence analysis

To examine the relationship among haplotypes, we constructed a minimum spanning tree (Rohlf 1973) based on the number of substitutions in pairwise comparisons. We used analysis of molecular variance (AMOVA, Excoffier et al. 1992) to examine the amount of genetic differentiation within and among local and regional population groupings. To determine whether the natural sampling locations of DFS were genetically subdivided we attempted various groupings. In AMOVA, the statistic F_{CT} measures the correlation of random genotypes within populations

relative to those drawn from all populations and measures the proportion of variation among groups of populations. The significance of F_{CT} is determined by comparison to distributions of F_{CT} values from 1000 random permutations of sequences among populations. The final groupings used in the analysis were those that maximized F_{CT} and were significantly different from random distributions. All of the above-mentioned analyses were performed using Arlequin 2.0 (Schneider et al. 2000).

Results

Microsatellites

Variation among subspecies

Of the ten optimized primer sets, seven loci were monomorphic in DFS and SFS (Table 2). For the seven monomorphic loci, the resulting allele size was the expected size based on the clone sequence. A minimum of 30 individuals were screened before determining that a locus was monomorphic. The number of alleles at the three polymorphic loci ranged from 7 to 12; however, there are only one or two common alleles for each locus (Figure 2). All three polymorphic loci were found to be in Hardy-Weinberg equilibrium. Overall, the allele distributions strongly overlap in SFS and DFS. There are eight alleles unique to SFS and ten unique to DFS, but these tend to be the more rare alleles. Based on randomization tests there was no significant difference in observed heterozygosity between SFS (H_o = 0.460) and DFS natural sampling locations (H_o = 0.423).

Variation within Delmarva fox squirrels

For all three loci, the most common allele is the same in the translocated and natural sites (Figure 2). Combining all loci, there are five alleles in the translocation sites that were not found in the natural range, but only one allele was unique to the natural range. Observed heterozygosities ranged from 0.083 to 0.611 (Table 3) and did not differ significantly between natural (0.423) and translocated (0.504) sites. However, allelic richness was significantly lower in the natural sampling sites (2.639) than in the translocation sites (3.447, p = 0.016).

Pairwise estimates of F_{ST} ranged from −0.0428 (between Kuehnle and TCF 3) to 0.2504 (between Whitemarsh and Harmony). Overall, pairwise F_{ST} values were higher among translocated than among natural locations and tests of population differentiation among the natural and translocated locations yielded many significant values (Table 4). However, of the comparisons among the seven natural DFS locations, only Kuehnle and Whitemarsh showed significant values of population differentiation.

Mitochondrial sequence data

Variation among subspecies

In a total of 184 individuals sequenced we detected 23 unique haplotypes (Table 5). There were 40 polymorphic sites with 30 transitions and 10 transversions. The average pairwise differences among haplotypes were similar within each subspecies, but the overall gene diversity was lower for DFS (Table 6). The nucleotide diversities (Nei 1987) within subspecies were similar and the average sequence divergence among subspecies was 2.93 (Table 6). Although haplotypes were not shared by the different subspecies, a minimum spanning network revealed that not all haplotypes from one subspecies clustered together (Figure 3). The two main clusters in the network show one that contains primarily DFS haplotypes and another primarily SFS haplotypes, but both include DFS, SFS, and WFS haplotypes. Two DFS haplotypes, (one and 11) cluster with six SFS haplotypes. Haplotype one is one of the four most common DFS haplotypes and is only three base pairs different from haplotype 10, the most common SFS haplotype. Similarly, SFS haplotypes 14 and 15 are more closely related to several DFS haplotypes and a WFS haplotype than they are to other SFS haplotypes. An AMOVA examining variation among DFS and SFS reveals significant ($p < 0.001$) among population variation with a V_a = 0.073 accounting for 15% of the variation.

Variation within Delmarva fox squirrels

Within DFS, we had samples from 10 natural locations and seven translocations, but we excluded the two natural locations with only one individual from our analyses. Overall there were nine DFS haplotypes. Haplotypes two, three, and four were the most frequent (0.224 for each) in the natural sampling sites and haplotypes one and two were the most frequent (0.258) in the translocation sites. We used AMOVA to determine if there was any population structure within the natural range. The analysis showed that a large majority of the variation is within populations. However, when we consider Jarrett East and Homeport

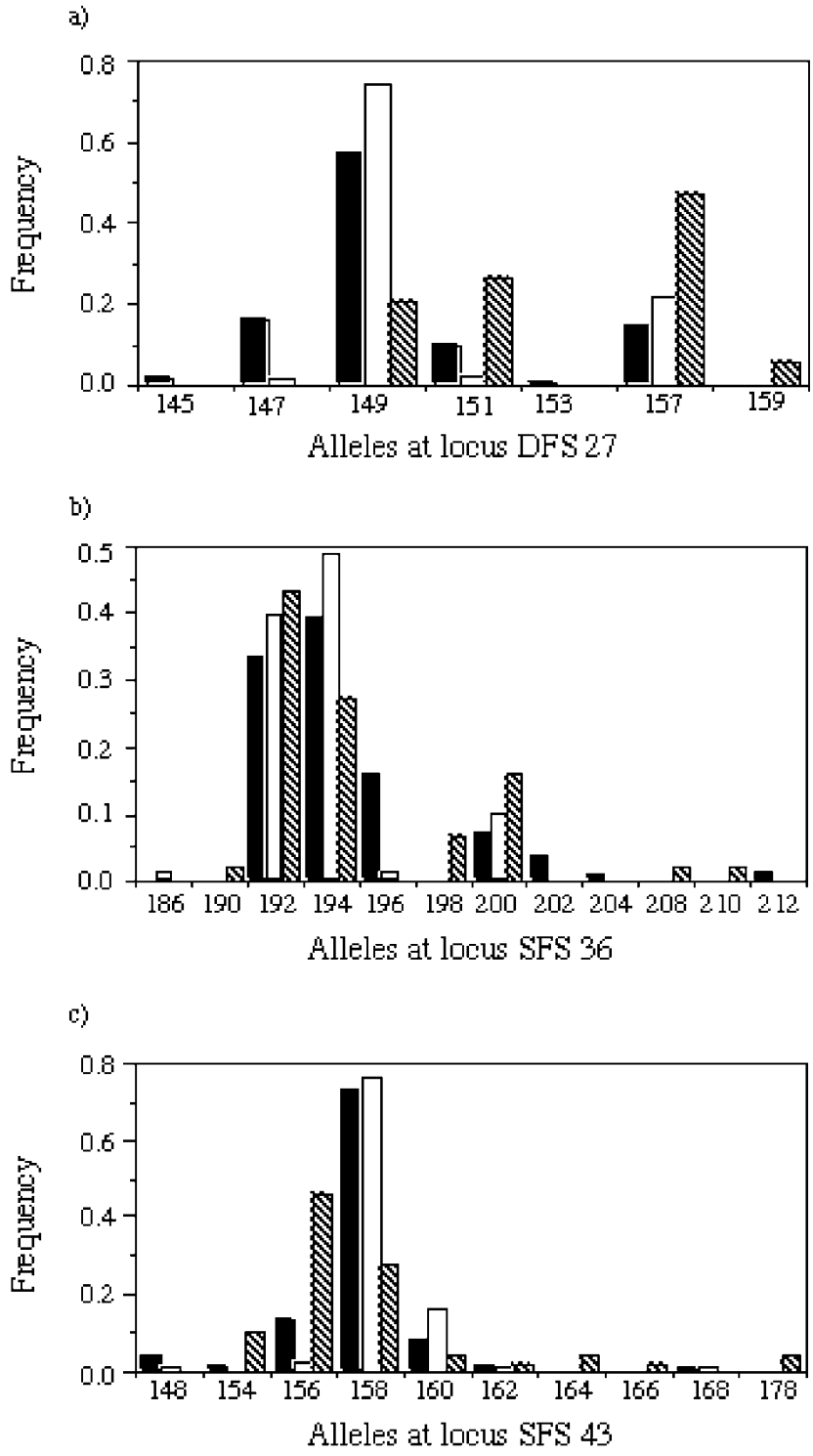

Figure 2. Allele frequency distributions at microsatellite locus (a) DFS 27, (b) SFS 36, and (c) SFS 43 for *S. n. cinereus* natural populations (open bars), translocated populations (solid bars), and *S. n. niger* (hatched bars).

Table 3. Allelic richness (Rs) at each polymorphic locus and observed (H_o) and expected (H_e) heterozygosity at three polymorphic loci for natural and translocated populations of DFS and for SFS. The Remington Farms population had only one allele at each locus and He was not calculated. Rs was not calculated for populations with fewer than 5 genotyped individuals

Population	RS SFS 36	RS SFS 43	RS DFS 27	H_o	H_e
DFS: Natural					
Jarrett West	3.480	2.873	2.239	0.470	0.471
Kuehnle	2.269	2.496	2.994	0.382	0.468
Jarrett East	2.976	1.967	1.998	0.423	0.410
White Marsh	3.867	2.538	1.538	0.378	0.396
TCF1	2.995	1.921	2.634	0.508	0.429
TCF2	2.875	2.875	2.000	0.429	0.466
TCF3	2.000	4.000	2.875	0.429	0.474
Homeport	n/a	n/a	n/a	0.083	0.535
DFS: Translocated					
CNWR	2.938	2.77	3.406	0.505	0.538
Eby	3.526	2.955	3.405	0.515	0.607
Harmony	3.873	2.778	4.789	0.417	0.569
Andelot	3.948	3.273	3.710	0.569	0.644
Quaker Neck	n/a	n/a	n/a	0.611	0.616
Dryden	n/a	n/a	n/a	0.278	0.421
Remington Farms	n/a	n/a	n/a	0.000	n/a
SFS	4.834	4.974	2.878	0.520	0.738

to be their own groups and combine the remaining six locations into a third group, the analysis does reveal significant among group variation (Table 7). Nevertheless, the among group variation explains only 18% of the overall variation, while variation within populations explains 79%.

Discussion

We examined three polymorphic microsatellite loci and sequenced part of the mtDNA control region. Overall, we found similar levels of genetic variation between DFS and SFS, and between the natural and the translocated range of DFS using nuclear and mitochondrial DNA markers. We recommend the continued use of translocations as a component of the DFS recovery effort.

Microsatellite data

Both DFS and SFS exhibited low levels of microsatellite polymorphism. We found only three polymorphic loci after screening a panel of 10 microsatellite loci designed for both subspecies. This low number of polymorphic loci does not allow us the power to conduct many statistical analyses; however they yielded interesting results. Our heterozygosity values were similar between DFS and SFS and between translocated and natural sampling sites. In addition they concur with previous allozyme studies (Moncrief 1993; Moncrief and Dueser 2001). Almost all of the pairwise tests of population differentiation involving natural sampling locations were non-significant which was expected given that DFS movements have been recorded between the TCF1, TCF2, TCF3, and Whitemarsh populations (Bocetti and Pattee, unpublished data). Most important for this study is the comparison between translocated and natural locations. The observed heterozygosities were similar in translocated and natural locations and we did not find a loss of alleles in the translocation sites. Rather, allelic richness was higher in the translocation sites (Table 3). As mentioned above, many of the translocations were established with squirrels from multiple sources, which may explain the increase in allelic richness. Only one allele was unique to a natural location, whereas five were unique to translocation sites. Overall, there was little difference between the sampled translocated and natural sites.

Mitochondrial sequence data

Variation among subspecies

MtDNA haplotypes were not shared among subspecies. However, based on the minimum spanning network it appears that some haplotypes from one subspecies are more closely related to those from other subspecies, than to their own. Haplotype 1 is one of the four most common haplotypes within DFS, yet it differs by only three base pairs from one of the most common haplotypes found in SFS. Given the relationship among the haplotypes, it was surprising not to find any that were shared. We believe we have sampled extensively from the range of DFS, but further sampling of SFS may yield haplotypes that are shared with DFS.

The precise historic ranges of DFS, WFS, and SFS are poorly known. It is likely that the ranges of DFS, SFS, and WFS abutted as recently as the 1800s (Bailey 1946). SFS occurred throughout southern Virginia in the early 1900s (Linzey 1998), was known in Chesterfield and Prince George's Counties in 1945 (Handley and Patton 1947), and is recently known only in

Table 4. Matrix of F_{ST} values among four translocated and seven natural sampling sites of *S. n. cinereus* and the pooled sampling sites of *S. n. niger*. Asterisks indicate significance at the $p < 0.05$ level for pairwise tests of population differentiation with standard Bonferroni corrections

	CNWR	Eby	Harm.	And.	Jarrett West	Kuehnle	Jarrett East	White-marsh	TCF2	TCF3	TCF1	*S. n. niger*
CNWR	–											
Eby	0.1338*	–										
Harmony	0.0586*	0.1617*	–									
Andelot	0.1094*	0.0430	0.1249*	–								
Jarrett West	0.0242	0.0918*	0.1282*	0.1435*	–							
Kuehnle	0.0136	0.1120*	0.1007*	0.1217*	0.0175	–						
Jarrett East	0.0762*	0.0633*	0.1949	0.1274*	0.0099	0.0326	–					
Whitemarsh	0.1435*	0.0720	0.2504	0.1508*	0.0557	0.1276*	0.0320	–				
TCF2	0.0330	0.0786	0.1374*	0.1104	−0.0106	0.0132	0.0210	0.0012	–			
TCF3	−0.0021	0.0839*	0.0772	0.0887	−0.0112	−0.0428	0.0134	0.0960	−0.0223	–		
TCF1	0.0106	0.0886*	0.1137	0.1274*	−0.0228	−0.0102	−0.0102	0.0835	0.0080	−0.0268	–	
S. n. niger	0.1161*	0.1945*	0.1674*	0.1188*	0.1869*	0.1464*	0.2011*	0.2646*	0.1667	0.1270	0.1738*	–

Table 5. Sciurus niger cinereus, *S. n. niger*, *S. n. vulpinus* and *S. n. rufiventer* haplotype distributions in the 26 sampling localities

	Haplotypes																						
Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
S. n. cinereus																							
Translocations																							
Andelot	4	1		1							7												
CNWR		8	8	5																			
Eby Farm	9																						
Quaker Neck	1																						
Dryden	1				2																		
Harmony	1	5			5						1												
Remington Farms		2		1																			
S. n. cinereus																							
Natural pops.																							
Jarrett West	3	5	5	4		3																	
Kuehnle	7	1	5	6	1	1																	
Jarrett East		7		2		2	1																
Whitemarsh	2		5	1	2			1															
TCF 1	2	3		6																			
TCF 2	3		2	3																			
TCF 3	2	2	4																				
Homeport											2												
Chino Farms			1																				
Hayes Farm		1																					
S. n. niger																							
Spring Island									2	5									1				
Allendale												2		1				1					
Garnett														2		1							
Twickenham															1					1			
Yemassee									1								1						
Unknown in SC									1	2													
S. n. vulpinus																							
Ft. Defiance																					1		1
Rte 257/Rte 613																						1	
S. n. rufiventer																							
Westwood													1										

Table 6. Sequence statistics for three subspecies of fox squirrel for a 330 bp fragment of the mitochondrial control region. Diagonal numbers in italics: Nei's (1987) nucleotide diversity within sub-species. Lower left hand corner: average sequence divergence among subspecies

	S. n. cinereus	*S. n. niger*	*S. n. vulpinus*
S. n. cinereus	*0.021*	–	–
S. n. niger	0.027	*0.019*	–
S. n. vulpinus	0.033	0.028	*0.031*

Amelia and Surrey Counties (Dueser and Handley 1991). WFS are common to the west of the Blue Ridge Mountains in Virginia, but occur spottily to the east (Fies 1993). It was also found in counties in eastern Maryland, but on the western shore of the Chesapeake Bay as recently as the 1940s, (Mansueti 1948) and is still present in western and southern Pennsylvania. DFS historically occurred in Accomack and Northhampton Counties on peninsular Virginia (Linzey 1998) and occurred in southern Pennsylvania as recently as 1906 (Poole 1932). Thus, the geographic isolation of these three subspecies is recent and the current pattern of mtDNA variability likely reflects incomplete lineage sorting.

We found similar levels of sequence divergence between the two southeastern subspecies (DFS and SFS) as between either southeastern subspecies and the western subspecies (WFS). In order to elucidate the phylogenetic relationships of DFS to other subspecies of fox squirrels, we recommend that a phylogenetic study be undertaken on the entire *S. niger* group. Currently, two subspecies, *S. n. shermani* and *S. n. avicennia*, are listed on the Florida state endangered or threatened species lists (Loeb and Moncrief 1993) and the status of SFS is of concern (Wood and Davis 1981; Kammermeyer 1994). A more complete understanding of the relationships among subspecies may be helpful if future translocations are necessary to protect the species.

Variation within DFS

The results of the AMOVA showed that two of the sampling locations, Jarrett East and Homeport, within the remaining natural range of DFS, are slightly differentiated from the others. This result may be due to two common haplotypes, one and three, not being found in Jarrett East and haplotype seven being unique to Jarrett East. Similarly, Homeport is the only natural location where haplotype 11 occurred. This haplotype was found in two translocation sites (Andelot and Harmony). Both of these sites received squirrels from Queen Anne's County in which Homeport is located.

Management implications

The DFS has been listed as endangered since 1967. Habitat loss continues to be a serious threat on the Delmarva Peninsula. In general, increasing population sizes and repopulating areas in the historic range are better accomplished by translocating wild animals than by reintroducing captive reared animals (Snyder et al. 1996). We found similar levels of genetic diversity in the examined translocated and natural sites. The translocation sites are only 20 to 30 years old, are not likely to have reached equilibrium, and may still lose genetic variation. In general, our data for the translocation sites indicate that the founding event itself did not create a loss of allelic diversity or heterozygosity. Our main objective is to maintain or to increase the amount of genetic variation in the natural and translocated locations of DFS and our management recommendations stem from this goal.

Our primary recommendation is to continue using translocations as a management tool for expanding the range of DFS. The strategy of moving squirrels from multiple source areas should also be maintained. However, decisions will need to be made concerning which source locations to use. If translocations were being established as a new recovery strategy it would be recommended to mimic natural dispersal. Studies document dispersal of approximately 1.5 km in DFS (Paglione, pers. comm.). Due to the extreme reduction in range and concentration of DFS into several counties, most of the translocation sites were well beyond the natural dispersal distance of the natural range and mimicking dispersal was not an option. Animals from the natural range in Queen Anne's County possess a haplotype not found in Dorchester County. However, our sample size is insufficient to make any conclusions regarding substructuring between northern and southern regions. Some translocation sites received squirrels from both Queen Anne's and Dorchester Counties. Others that are further north than Queen Anne's County were established with squirrels from Dorchester County. Thus, maintaining separation between the northern and southern regions is not possible. Rather, we recommend continuing to use squirrels from both counties to avoid losing the variation found in Queen Anne's County.

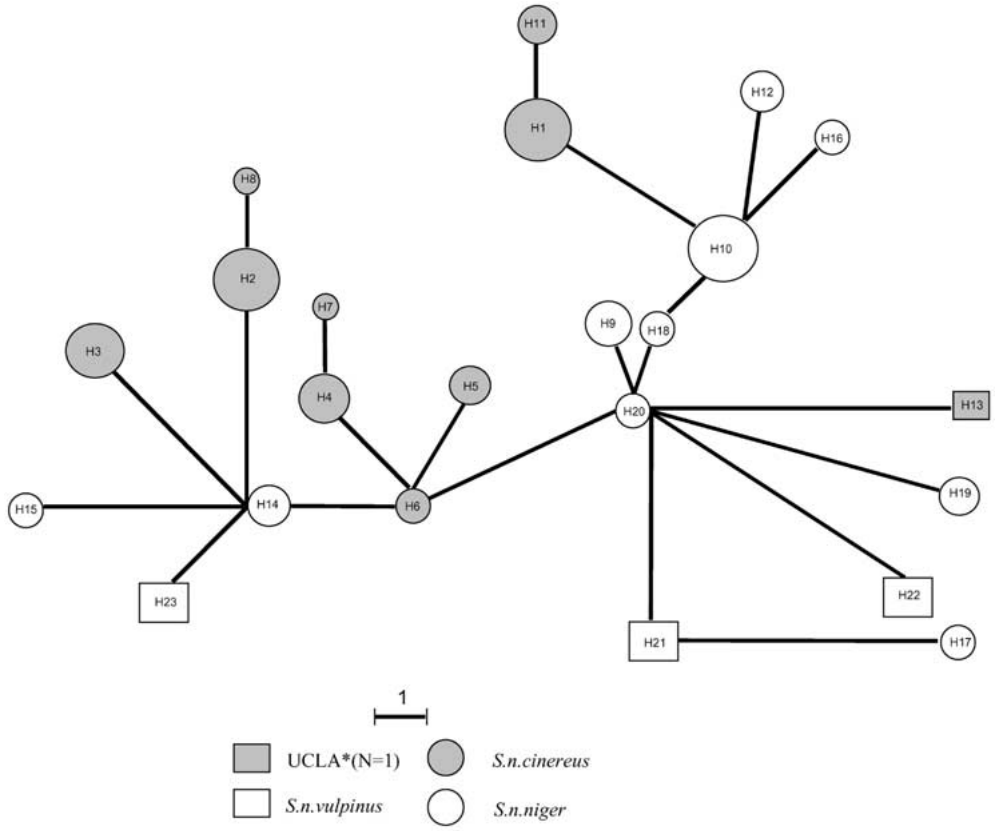

Figure 3. Minimum spanning network tree based on the number of substitutions among control-region haplotypes. Sizes of symbols are proportioned to the relative frequency of haplotypes within subspecies.

Table 7. Results of an AMOVA showing the partitioning of the genetic variation of natural populations of DFS when Jarrett East and Homeport are considered separate populations from the remaining five sampling localities

Source	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among groups	2	3.05	0.089 Va	18.63
Among populations within groups	5	2.58	0.011 Vb	2.37
Within populations	85	31.725	0.373 Vc	79.00
Total	92	37.355	0.472	

Overall, the translocation sites we sampled appear to reflect the levels of genetic diversity in the natural range. The distribution range of DFS has been substantially reduced, and it is unknown whether the variation observed represents a reduction from its historic levels. The few differences that we see among sampling locations are not likely to reflect adaptive differences. Thus, the translocations appear to be maintaining current levels of variation and should be continued as a conservation strategy.

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