Eastern Illinois University
Department of Biological Sciences

(January 1, 2006 – December 31, 2007)

Ecology and Genetics of Red Squirrels in Illinois

Final Project Report 2008

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Project Title: Ecology and Genetics of Red Squirrels in Illinois

EXECUTIVE SUMMARY

The North American red squirrel (*Tamiasciurus hudsonicus*) is common throughout much of its geographic range and the biology and ecology of this species have been studied extensively. The present range of the red squirrel in North America encompasses much of Canada, New England, the upper Midwest, as well as the Rocky Mountains and Appalachian Mountains. Although red squirrels are commonly associated with coniferous forests, they also occupy the fragmented landscapes of northern Indiana, Ohio, and Illinois. Here deciduous forests, pine plantations, and fencerows provide habitat for the species.

In Illinois, the history and ecology of the red squirrel are poorly understood. The species likely inhabited the northern one-third of the state in the early 1800s, but disappeared ~1900. In 1977, a population of unknown origin was identified in Kankakee Co. in northeastern Illinois. Speculation regarding the source of this population prompted four hypotheses: (1) red squirrels may have been translocated from Minnesota, (2) a remnant population may have persisted in the area unnoticed until the 1970s, (3) squirrels may have immigrated naturally from Indiana in the 1970s, or (4) the population might be some combination of descendents from translocations, immigration, and/or native squirrels. Prior to this study, the origins, and therefore the conservation status, of Illinois' red squirrel population was unclear and controversial. The species is currently considered to be a conservation priority mammal as defined in the Illinois Wildlife Action Plan. Consequently, this study was conducted to investigate the: (1) genetic origin of Illinois' red squirrels, specifically whether they represent a unique genetic legacy, (2) current distribution of the species in the state Illinois, including whether the population appears

to be expanding, stable, or declining, and (3) use of habitat and resources, specifically those necessary to conserve red squirrels in Illinois. Field surveys, interviews, intensive telemetry studies, and genetic analyses commenced in March 2006 and continued through December 2007.

To address the origin of Illinois' red squirrel population, genetic analyses were conducted on tissues from 229 squirrels from five Midwestern states (IL, IN, MI, WI, and MN), including 52 Illinois red squirrels. Our approach was to compare the genetic composition of Illinois' squirrels to that of other states' populations to infer the likelihood that Illinois' squirrels originated from each state. We isolated, amplified, and sequenced loci from both mitochondrial DNA (mtDNA) and nuclear DNA microsatellites, then used program STRUCTURE to estimate probabilities of population origin and program GENELAND to investigate more broadly the genetic structure of Midwestern red squirrel populations. Independent evidence from both mtDNA and microsatellites indicated that Illinois' red squirrel population originated primarily from immigrants from Indiana and lower Michigan. However, it is also likely that a one-time translocation of squirrels from Minnesota contributed to the present population. Microsatellite analysis indicated that the Illinois population differs from others in the region, not because it includes unique alleles, but rather because it includes the genetic signature of both natural immigrants from the east and translocated squirrels from Minnesota. This unique situation has produced an admixed population in Illinois. We found no evidence that Illinois' squirrels constitute a genetically-unique remnant population.

Throughout 2006 and 2007, we conducted extensive personal interviews and audio-playback surveys in the field to delineate the current distribution of red squirrels in Illinois and compare past and present ranges to evaluate whether the species' range is expanding, restricting,

or stable. Our results showed that red squirrels occupy a broader geographic range than previously known, extending ~50 km farther to the southwest and ~30 mi to the northwest than in 1980. However, the northern perimeter of the range has constricted by ~20 km, perhaps due to increasing urbanization and expansion of the south Chicago suburbs. We speculate that red squirrel populations may be slowly expanding in the state. Agricultural fields, grasslands, roadways, and rivers do not appear to be barriers to movement. Range expansion is occurring primarily (but not exclusively) along the riparian forests of the Kankakee and Iroquois Rivers. We also found the species in small, isolated woodlots, including one at Goose Lake Prairie surrounded by more than 1,000 ha of prairie and >1.5 km from the nearest forested corridor.

Red squirrels inhabit deciduous forests, both with and without a coniferous component, in Illinois. The understory of inhabited woodlots varied from sparse to very dense. However, two factors were common to all occupied woodlots: (1) the presence of mature walnut trees, and (2) the absence or scarcity of gray squirrels. Occupied patches varied from 5 ha to >1,500 ha in size and from narrow to wide in shape. We found no evidence that patch size or shape influenced occupancy. Woodlots <20 ha in size were occupied suggesting that the species is not particularly area-sensitive, as were patches >2 km from the nearest apparent habitat indicating that they are capable of dispersing across stretches of non-forested landscape.

During the summers of 2006 and 2007, we conducted an intensive telemetry study at Iroquois County State Wildlife Area to investigate habitat use and the spatial ecology of red squirrels. Summer home ranges averaged 1.7 ha (SE = 0.24) in size and core areas averaged 0.4 ha (SE = 0.06). The size of home ranges and core areas did not differ between males and females or adults and juveniles. Home ranges are generally larger than those reported for red

squirrels inhabiting prime habitats. We interpret this to mean that northeastern Illinois provides only marginal habitat quality for this species. Individuals of both sexes spent considerable time on the ground foraging in thickets and dense understory. We found little evidence of territorial behavior in this population; home ranges and core areas frequently overlapped and middens of pinecones or walnuts were not observed. These squirrels appear to be non-territorial scatter-hoarders.

Relative to the composition of the study area as a whole, home ranges were comprised predominantly of deciduous forest and pine plantations. Grasslands and agricultural fields were avoided. At the finer scale of habitat use within home ranges, we found no clear preferences among forest types. X^2-tests suggested that squirrels preferred brush-savanna and pine plantations to deciduous forests, but compositional analysis ranked brush-savanna as the most preferred, followed by deciduous forest, then pine plantation. Field observations showed that mature walnut trees and a dense understory are important habitat components for these animals. Gray and fox squirrels were not abundant in forests used by red squirrels, but chipmunks are likely competitors for summer food and burrows. Our results suggest that red squirrels are not dependent on pines and are likely to persist in this region in spite of the ongoing removal of non-native pine trees provided that mast-producing walnuts and dense understory vegetation are present.

In summary, we found little evidence that the red squirrel should be considered as a species in need of special protection in Illinois. Their genetic composition is similar to squirrels in neighboring states and suggests that the population was founded primarily by immigrants from Indiana. This population is therefore at the western extent of the species' range. Field surveys

indicate that the species has expanded, albeit slowly, through habitat that is fragmented and of marginal quality. Management practices that serve to maintain riparian corridors, retain mature mast-producing hardwoods (particularly walnuts), and provide dense understory thickets will help to conserve red squirrels. Whereas non-native pine plantations are used by squirrels in northeastern Illinois, the presence of red squirrels in deciduous forests lacking pines suggests that the removal of these plantations on public lands does not threaten the persistence of red squirrels in the region.

JOB 1. INVESTIGATE THE GENETIC LINEAGE OF THE ILLINOIS RED SQUIRREL POPULATION

INTRODUCTION

The North American red squirrel (*Tamiasciurus hudsonicus*) is common throughout much of its geographic range and the biology and ecology of this species have been studied extensively. Topics as diverse as genetics (Arbogast et al. 2001; Wilson et al. 2005), coevolution with conifers (Parchman and Benkman 2002), competition with other granivores (Benkman et al. 2001), and edge effects (Anderson and Boutin 2002) have been addressed in recent years. The present range of the red squirrel in North America (Figure 1) encompasses much of the Rocky Mountains, Appalachian Mountains, and New England. Although red squirrels are commonly associated with coniferous forests, they are known to occur in the heavily fragmented landscape of the Midwest, including Indiana and Ohio (Steele 1998). The pine plantations, fencerows, and mixed forest patches characteristic of the Midwest are considered marginal habitat for the species (Yahner 2003). Despite this, red squirrels have persisted in areas such as Indiana throughout the 20th century (Lyon 1936).

The history and ecology of the red squirrel in Illinois are poorly understood. It likely inhabited the northern portion of the state in the early 1800s, but disappeared circa 1900 (Hoffmeister 1989). In 1977, a population of unknown origin was identified in Kankakee Co. in northeastern Illinois. Shortly thereafter, red squirrels were confirmed in Will Co. to the north and Iroquois Co. to the south (Hoffmeister 1989). Speculation regarding the geographic source of the newly discovered red squirrel population in Illinois prompted four hypotheses. First, several accounts suggest red squirrels from Minnesota

were released near Iroquois Co. State Wildlife Area (ICSWA) in the 1970s establishing the present population (Hoffmeister 1989). Second, a small native population of red squirrels may have persisted in the area through the early 1900s and went unnoticed until the 1970s. Third, as advocated by Hoffmeister (1989), red squirrels may have immigrated naturally from Lake and Newton Cos. in Indiana in the 1970s. Consistent with the immigration hypothesis is the expansion of red squirrels into the Willow Slough Fish and Wildlife Area, approximately 2 km east of ICSWA, in the 1970s (Mumford and Whitaker 1982). Finally, the current population might be a combination of the descendents of translocated squirrels from Minnesota, remnant Illinois individuals, and/or immigrants from Indiana.

In an earlier attempt to address the origin of Illinois' current red squirrel population, Brown and Edwards (1985) conducted a morphological analysis of 14 skulls from Illinois. Based on this analysis, 7 skulls were assigned to the group native to Indiana and Ohio, and 6 skulls were assigned to the type that occurs in Minnesota, Wisconsin, and northern Michigan. One skull was not assigned to either source due to missing data. Brown and Edwards (1985) offered two, mutually exclusive interpretations of the data: 1) the current population is of mixed ancestry or 2) low correlations in the analysis indicate insufficient data to discriminate between the two subspecies.

The Illinois Department of Natural Resources (IDNR) has designated the red squirrel as a critical species in need of conservation (IDNR 2005) based on its limited geographic range and low numbers in the state. Presently, the red squirrel is protected in Illinois, but not listed as threatened or endangered. Consequently, this study was conducted in large part to elucidate whether there is genetic evidence suggesting that the population is a unique genetic remnant population in need of protection, and if so to

provide the necessary baseline ecological information to scientifically conserve and manage the species in Illinois. In order to include a species on the Illinois Endangered and Threatened Species List, at least one of six conditions must exist (IDNR 2006). The red squirrel does not qualify for three of the conditions because (1) it is not listed as federally endangered or threatened, (2) it is not a candidate for listing as federally endangered or threatened, and (3) it does not exhibit a restricted geographic range across the continent. However, prior to our study, the species apparently met two of the three remaining conditions for listing: (1) it was once widespread in Illinois, but now exhibits a restricted range due human actions, (2) it exhibits restricted habitats or low populations in Illinois, and it may constitute a population geographically or genetically disjunct from other populations.

An important focus of this study was to investigate the phylogeogeographic origin of the Illinois red squirrel population. Specifically, our *a priori* viewpoints were that if the population is composed of descendents of translocated squirrels from Minnesota, then the population should be considered exotic and in no need of protection. However, if these squirrels are a remnant population with a unique genetic legacy, disjunct from the rest of the Midwest, inclusion on the Illinois endangered and threatened species list would be warranted. Finally, if the population is comprised mainly of descendents of recent immigrants from Indiana, and surveys showed that the population is expanding its geographic range in the state, then no special management efforts may be necessary and limited financial resources might better be devoted to other species in greater need of protection.

Modern molecular and genetic techniques allow the questions regarding the geographic origin of Illinois' red squirrels to be examined from a contemporary perspective. Arbogast et al. (2001) and Wilson et al. (2005) demonstrated the utility of genetic techniques, examining relationships among red squirrel populations across historic, geographic barriers, particularly rivers and mountain ranges. Arbogast et al. (2001) analyzed the mitochondrial cytochrome-*b* gene in red squirrels and used parsimony analysis to suggest two distinct clades, one confined to southern Colorado, New Mexico and Arizona and another clade spanning the rest of North America. Additionally, the control region [Displacement(D)-loop] in mitochondrial DNA (mtDNA) indicated a phylogenetic split on opposite sides of the Green River in Wyoming (Wilson et al. 2005). Thus, all squirrels collected in this study represent a single clade based upon these data.

The heavily fragmented landscape of the Midwest poses a different challenge for studying the genetics of forest-dwelling taxa. While there are few topographic barriers to gene flow, large expanses of unsuitable habitat, including agricultural fields, roads, and urban areas, may isolate red squirrel populations. For example, the current geographic range of the red squirrel indicates a disconnect between the Wisconsin and Illinois populations (Hanson 2008). Therefore, the primary objectives of this portion of our study were to: (1) elucidate the geographic origin of the current red squirrel population in Illinois utilizing mitochondrial and microsatellite DNA, and (2) examine patterns of genetic variation in the red squirrel across the Midwest.

METHODS

Sample Collection

In Illinois, tissue samples were taken with a 2-mm ear punch from anesthetized live-trapped individuals as part of the concurrent telemetry study (Hanson 2008). Handling and sampling protocols followed the guidelines of the American Society of Mammalogists (Gannon et al. 2007) and were approved by Eastern Illinois University's Animal Care and Use Committee (Permit EIU06010). Researchers, trappers, and hunters provided tissue samples from harvested squirrels in all other Midwestern states. Samples were initially preserved in 100% ethyl alcohol or desiccant beads and transferred to an ultra-low freezer (-60°C) for long-term storage.

Laboratory Methods and Data Analysis

Mitochondrial DNA. - The mtDNA analyses included 229 tissue samples collected from red squirrels inhabiting five Midwestern states, including 52 from Illinois, 50 from Indiana, 43 from Michigan, 52 from Minnesota, and 32 from Wisconsin (Figure 2). Samples from the Upper Peninsula (UP) of Michigan were grouped with Wisconsin squirrels in final data analyses due to geographic proximity and a preliminary analysis that indicated the Michigan UP shared more haplotypes (3) with Wisconsin than it did with lower Michigan (0). The corrected totals taking into account the re-grouping are: Illinois 52; Indiana 50; Michigan 24; Minnesota 52; Wisconsin 51. The methods and results discussed henceforth for mtDNA were based upon this latter grouping.

Whole genomic DNA was extracted using the DNeasy Tissue Kit following the protocol provided by the manufacturer (Qiagen, Valencia, California) or a modified ammonium acetate protocol (Latch et al. 2008). A 272 - 274 base pair section in the

hypervariable domain III of the non-coding control region (D-loop) was amplified with polymerase chain reaction using the primers OSU5020L (5'-CCTTTAGCTGGCATAG GTA-3') and OSU5021H (5'-CATTATATGGAGTGGAGAA GG-3'; Wilson et al. 2005). Each 25 μl reaction included 10 – 50 ng of genomic DNA, 2.5 μl 10× buffer, 2.5 mM $MgCl_2$, 1.5 μM of each primer, 0.08 mM of each dNTP (Qiagen) and 0.5 units of *Taq* DNA polymerase (Qiagen). The PCR profile was 94°C for 3 minutes followed by 35 cycles of 94°C for 45 seconds, 54°C for 30 seconds and 72°C for 1 minute with a final elongation of 72°C for 10 minutes. PCR products (5 μl) were electrophoresed through a 1% agarose gel, stained with ethidium bromide and evaluated with ultraviolet light. Successful samples were purified with the Wizard PCR Prep DNA Purification System (Promega, Madison, Wisconsin) and sequenced on an ABI 3730XL with Big Dye 3.1 cycle sequencing chemistry (Applied Biosystems, Foster City, California) at the Purdue University Genomics Core Facility. Samples were sequenced with the forward and reverse primers. All sequences were manually edited in the computer program SEQUENCHER v.4.7 (Bromberg et al. 1995).

Sequences were aligned in CLUSTALX (Thompson et al. 1997) with the default multiple sequence alignment parameters (Gap opening, 10; gap extension, 0.2; DNA Transition Weight, 0.5). The shape parameter of the gamma distribution was calculated based on a neighbor-joining tree constructed using the program DAMBE v.4.5.57 (Xia and Xie 2001). Nucleotide diversity (π) and haplotype diversity (h) were calculated in ARLEQUIN (v.2.0; Schneider et al. 1997) by state. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) with 16000 permutations was performed in ARLEQUIN based on genetic distances among haplotypes (Tamura and Nei 1993). The AMOVA

procedure examined nucleotide diversity within and among states and was used to calculate Φ-statistics between states. Significance was determined with 3000 permutations in ARLEQUIN and the false discovery rate (FDR; Benjamini and Yekutieli 2001) was utilized to obtain an experiment-wide alpha level (α_{EW}) to account for multiple tests. This method offers increased statistical power compared to the conservative Bonferroni correction (Rice 1989).

To test the hypothesis of population expansion in the Indiana and Illinois populations, Fu's F_S (Fu 1997) and a mismatch distribution (Rogers and Harpending 1992) also were performed in ARLEQUIN. An unrooted haplotype cladogram was generated in TCS (v.1.21; Clement et al. 2000). A 95% connection limit was applied and gaps were considered a 5th state in the analysis. The cladogram was edited to illustrate the frequency and location of each haplotype.

Microsatellite DNA. - The microsatellite analyses included 232 red squirrel tissue samples, with 52 from Illinois, 46 from Indiana, 43 from Michigan, 56 from Minnesota, and 35 from Wisconsin (Figure 2). Samples from the Michigan UP were grouped with Wisconsin squirrels due to geographic proximity and comparative purposes. The re-grouped sample sizes were: Illinois 52; Indiana 46; Michigan 24; Minnesota 56; Wisconsin 54. The methods and results discussed henceforth for microsatellite DNA were based upon this latter grouping.

Six microsatellite loci (Thu03, Thu21, Thu25, Thu33, Thu37, Thu42; Gunn et al. 2005) were amplified separately with PCR. Each 10 μl reaction included 10 – 50 ng of genomic DNA, 2 μl 5× buffer, 2.5 mM $MgCl_2$, 0.6 μM of each primer, 0.08 mM of dNTPs (Promega, Madison, Wisconsin) and 0.2 units of *Taq* DNA polymerase (Promega). The

PCR profile was 94°C for 2 minutes followed by 30 cycles of 94°C for 1 minute, annealing temperature (between 58°C and 64°C) for 30 seconds and 72°C for 30 seconds with a final elongation of 72°C for 10 minutes. The forward primer was labeled with the D4 WellRED fluorescent tag (Sigma-Aldrich, St. Louis, Missouri) and products were electrophoresed through a high-resolution polyacrylamide gel on a Beckman CEQ 8000 (Beckman-Coulter, Fullerton, California). Allele sizes were determined using the Fragment Analysis v.2.3.4 software (Beckman-Coulter). Samples with low signal intensity were amplified and electrophoresed until a satisfactory signal was observed. An additional 25 unknown samples were also amplified and electrophoresed to quantify error for each locus.

The computer program CONVERT (v.1.2, Glaubitz 2004) was utilized to format data for input into the various software packages. We tested for a deficiency of heterozygotes in the entire population in GENEPOP (v.4.0, Rousset 2007) for each locus and globally (Rousset and Raymond 1995). To estimate *p* values, the Markov chain method of Guo and Thompson (1992) was utilized (dememorization steps = 10000, number of batches = 500, iterations per batch = 5000). An unbiased probability of identity (PID) was also calculated to estimate the power of the loci utilized in the analyses (Paetkau et al. 1998).

The program GDA (v.1.1; Lewis and Zaykin 2001) was utilized to calculate observed and expected heterozygosity, average number of alleles, total number of unique alleles, and frequency of unique alleles for each state population. Pair-wise F_{ST} values were calculated in ARLEQUIN (v.2.0; Schneider et al. 1997) and standardized F_{ST} values (F'_{ST}) were calculated following the method of Hedrick (2005). The standardized measure (F'_{ST}) adjusts for the high level of variation observed in loci like microsatellites and ranges

from 0 to 1. The traditional F_{ST} may not range from 0 to 1 for loci with high levels of polymorphism (Hedrick 2005). Evidence of a genetic bottleneck in the Illinois population was tested with the mode shift method (Luikart et al. 1998) in the program BOTTLENECK (v.1.2.02; Piry et al. 1999). The mode shift method was utilized because alternative tests (sign test, Wilcoxon test) are sensitive to significant departures from Hardy-Weinberg equilibrium (Luikart and Cornuet 1998). The false discovery rate (FDR; Benjamini and Yekutieli 2001) was utilized to obtain an experiment-wide alpha level (α_{EW}) to account for multiple comparisons when appropriate (locus specific Hardy-Weinberg tests and F_{ST} pair-wise comparisons).

We used the computer program STRUCTURE (v.2.1; Pritchard et al. 2000) to test several hypotheses regarding population structure. To test the hypothesis of a remnant Illinois population, we estimated K, the number of populations, following the procedure outlined in Pritchard and Wen (2004) for $K = 1$ to $K = 10$ given all samples. In a subsequent analysis, Illinois samples were excluded for comparative purposes. We followed the procedure outlined in Evanno et al. (2005), using ΔK as an indicator of the number of clusters within our data to provide a better estimate of K. Each run consisted of a burnin of 30,000 followed by 100,000 replicates for 10 runs at each K.

A traditional assignment test (Manel et al. 2005) was performed in STRUCTURE to test the hypotheses of immigration from Indiana and translocation from Minnesota with all Illinois individuals serving as unknowns. Four potential source populations were defined *a priori* (Indiana, Michigan, Minnesota, Wisconsin). In this analysis, 10 runs were performed, each consisting of a burnin of 30,000 replicates followed by 100,000 replicates of the Markov Chain Monte Carlo (MCMC). We calculated the mean Q-vector for each

Illinois individual in the sample and an average for all Illinois squirrels. The maximum observed value in the mean Q vector for each individual was used for assignment to one of four source populations defined *a priori* (Indiana, Michigan, Minnesota, Wisconsin). An admixture model was applied and allele frequencies were allowed to correlate among populations (Falush et al. 2003) for all analyses performed in STRUCTURE.

GENELAND (v.2.0.12; Guillot et al. 2005b) may detect population substructure at lower levels of differentiation than STRUTURE due to its ability to include spatial information (Guillot et al. 2005a). In GENELAND both spatially explicit and implicit models were run. The UTM coordinates of each squirrel's location were recorded when possible and applied to the spatial models. In other cases, where only the county of origin for a tissue sample was known, the coordinates corresponding to the center of the county were used. In order to minimize the error associated with these coordinates, we ran spatially explicit models with uncertainty coordinates of 1 km, 10 km, 50 km and 100 km. A 1-km uncertainty coordinate was considered appropriate for samples from known locations due the known dispersal capabilities of juvenile red squirrels (Berteaux and Boutin 2000; Larsen and Boutin 1994). Uncertainty coordinates of 10 km, 50 km, and 100 km also were utilized to determine if GENELAND clustered the samples differently in these analyses and to account for the limited spatial information for some samples. The Dirichlet distribution (Pritchard et al. 2000) was used to model allele frequencies because it has been shown to perform better than the alternative F-model (Guillot et al. 2005a). The maximum rate of the Poisson process was set to 232, the number of individuals in the sample as recommended by Guillot et al. (2005a). The maximum number of nuclei in the Poisson – Voronoi tessellation was set to 696, three times the maximum rate of the Poisson

process, as also suggested by Guillot et al. (2005a). The various uncertainty coordinates were used along with 100,000 iterations of the MCMC and a thinning of 10 for a total of 10,000 stored replicates in analyses to infer the number of populations. Each model was run 10 times to obtain an accurate estimate of *K* given the data. Additionally, the MCMC was run 10 times without coordinates (Spatial Model = False) to compare results. In this analysis, the maximum number of nuclei was set to 232, the number of individuals. We did not observe a change in the results with longer runs of the MCMC (1,000,000 iterations, thinning of 10 for 100,000 stored replicates).

Results of the analyses in GENELAND with differing uncertainty levels were examined to determine the true number of populations, utilizing the mode of *K* as an estimate. We then performed 5 additional runs each for the uncertainty levels of 1 km, 10 km, 50 km, and 100 km with *K* fixed (at the mode of *K* as determined by previous analyses) to assign individuals to populations as recommended by Guillot et al. (2005b). In these analyses, the MCMC was run 100,000 times, with a thinning of 10. The posterior probability of population membership for each pixel of the spatial domain (100×100 pixels with a burnin of 100) was also calculated and averaged over the 5 runs. Individuals were unambiguously assigned population membership if the probability of population membership was greater than 0.7.

Observed and expected heterozygosity were calculated on the inferred populations (based on GENELAND results) in addition to the average number of alleles, total number of unique alleles, and frequency of unique alleles. Levels of differentiation among the inferred populations were quantified with F_{ST} and F'_{ST}. The program BOTTLENECK was used to perform the mode shift test on the inferred populations.

RESULTS

mtDNA

Alignment of a 272 – 274 bp section of the mitochondrial control region revealed 43 variable sites with 36 transitions, 6 transversions, and 2 insertion/deletion events. A total of 47 haplotypes was found among 229 red squirrels sampled across a five-state region in the Midwest. The number of haplotypes found in each state ranged from 6 in Illinois to 17 in Wisconsin (Table 1). Twenty-six haplotypes were observed in only one individual with an additional 8 haplotypes found in multiple individuals, but observed in only one state. Wisconsin did not share any haplotypes with Illinois or Michigan, whereas all other states shared at least one haplotype. Illinois shared three haplotypes with Michigan and one haplotype with Minnesota. Five haplotypes were shared by squirrels inhabiting adjacent states in three cases: Illinois and Indiana, Indiana and Michigan, and Wisconsin and Minnesota. The most common haplotype (A) was observed 28 times in two states, Minnesota and Wisconsin. The second most common haplotype (B) was observed in Illinois and Indiana a total of 27 times. The third most common haplotype (C) was found in 21 individuals in the states of Illinois, Indiana, and Michigan. Illinois was the only state that did not contain at least one unique haplotype. We found 4 unique haplotypes among Indiana squirrels, 6 in Michigan, 10 in Minnesota, and 14 in Wisconsin.

We observed high haplotype diversity and nucleotide diversity among Michigan squirrels, despite a small sample (Table 1). The lowest observed haplotype diversity was observed in Illinois with the second lowest nucleotide diversity (Table 1). An AMOVA revealed 72% of genetic variation was attributed to variation among individuals within states while 28% of genetic variation was attributed to differences among states. The

overall Φ_{ST} was significant with 16000 permutations in ARLEQUIN ($\Phi_{ST} = 0.28$, $p < 0.001$), providing evidence of population substructure. All pair-wise comparisons of Φ_{ST} values showed significant differences with 3000 permutations in ARLEQUIN, except the Michigan-Indiana comparison and the Michigan-Wisconsin comparison ($\alpha_{EW} = 0.017$; Table 2). Additionally, Φ_{ST} values suggest Illinois squirrels are genetically similar to the Indiana population while the Φ_{ST} comparison between the Illinois and Minnesota samples suggests less similarity (Table 2). The mean Φ_{ST} value for all Illinois pair-wise comparisons is the highest, indicating dissimilarity among the Illinois population and all others sampled. Results also suggested squirrels from Indiana and lower Michigan were not significantly different, nor were those from lower Michigan and Wisconsin (Table 2). The relationship between Indiana and Michigan may be attributed to geographic proximity and a lack of barriers to gene flow. The low value associated with the Illinois and Indiana comparison supports immigration as the primary source of the extant Illinois red squirrel population. Also, tests of neutrality and a mismatch distribution analysis performed in ARLEQUIN did not show any indication of population expansion.

The unrooted haplotype cladogram (Figure 2) revealed Illinois squirrels most frequently shared haplotypes with Indiana squirrels and to a lesser extent Michigan squirrels. Overall, 40% of Indiana squirrels shared a haplotype with at least one Illinois individual while 33% of Michigan squirrels shared a haplotype with an Illinois squirrel. Haplotype F, observed in a single individual from Illinois, was observed in 11 Minnesota squirrels, accounting for 21% of squirrels sampled from Minnesota. Haplotype J was the only haplotype observed in Illinois that was seen more frequently in Michigan than Indiana. The unrooted haplotype cladogram also revealed Illinois haplotypes are more

closely associated with Indiana and Michigan haplotypes with two exceptions (Figure 2). Haplotype J, situated near the center of the cladogram, was one mutational step away from haplotypes observed in Indiana, Michigan, Minnesota and Wisconsin but three mutational steps away from the closest Illinois haplotype. Similarly, haplotype F (observed in 11 Minnesota samples and 1 Illinois sample) was five mutational steps away from the nearest Illinois haplotype (J) but only one step away from haplotypes found in Indiana, Michigan, Minnesota, and Wisconsin. Michigan exhibited the largest difference between two specific haplotypes with 14 mutational steps separating haplotypes CC and C (Figure 3). Two haplotypes found in Wisconsin (M and JJ) were 13 mutational steps apart, whereas Indiana, Illinois and Minnesota all had haplotypes 12 mutational steps apart. Consequently, the Φ_{ST} comparisons, haplotype frequency distribution, and unrooted haplotype cladogram all support immigration from Indiana as the primary source of the current Illinois red squirrel population.

Microsatellite DNA

Missing data accounted for 1.1% of the total sample of 232 red squirrels while no samples were removed from the analysis. The number of alleles per locus ranged from 11 (Thu37) to 17 (Thu42) with a mean of 13.83. Analyses performed on the entire sample indicated a significant deviation of Hardy-Weinberg Equilibrium globally ($\alpha = 0.05$; $p <$ 0.00) attributed to disequilibrium at all six loci ($\alpha_{EW} = 0.02$; $p_{Thu03} < 0.00$; $p_{Thu21} < 0.00$; $p_{Thu25} = 0.01$; $p_{Thu33} < 0.00$; $p_{Thu37} = 0.01$; $p_{Thu42} = 0.01$). Expected heterozygosity levels ranged from 0.76 to 0.80, whereas observed heterozygosity levels ranged from 0.68 to 0.71 within each state (Table 3). Minnesota contained the highest average number of alleles per locus and Illinois contained the lowest. Similarly, Minnesota contained the most unique

alleles and Illinois contained the fewest. Estimates of F_{ST} and F'_{ST} indicate low levels of population substructure (Table 4). Illinois contained the highest mean values for both of the parameters and Michigan contained the lowest mean values. Results of the mode shift test in BOTTLENECK did not show evidence of a genetic bottleneck in any of the given state populations. The overall probability of identity, combined for all six loci was 6.75×10^{-9}.

Attempts to estimate the true *K*, the number of population clusters, in STRUCTURE yielded ambiguous results. In the analysis that tested the hypothesis of a remnant population (Illinois samples included), the mean natural logarithm of the probability of the data [L(K)] was highest at $K = 3$, but was also comparatively high at $K = 1$ and $K = 2$ (Figure 4a). Additionally, the plot of ΔK did not illustrate a dramatic increase at any *K*, possibly indicating $K = 1$ (Figure 4b). The analysis that excluded Illinois samples yielded similar results, indicating $K = 1$. The ambiguity of results on the true *K* in STRUCTURE seems to be attributable to low levels of population substructure as supported by F_{ST} and F'_{ST} (Table 4). STRUCTURE begins to detect the true *K* at F'_{ST} values above 0.28; values of 0.39 and above are required for STRUCTURE to assigned 97% of individuals to the correct population (Latch et al. 2006).

The results of the traditional assignment test in STRUCTURE, in which state boundaries were used to delineate potential source populations, indicated an admixed population in Illinois. The mean *Q*-vector for all Illinois individuals over 10 iterations was: Michigan 0.46; Indiana 0.26; Minnesota 0.12; and Wisconsin 0.08 with 30 squirrels assigned to Michigan, 16 Illinois squirrels assigned to Indiana, 6 assigned to Minnesota, and 0 assigned to Wisconsin. Most individuals were assigned unambiguously (> 0.50) to

one population (Michigan =24 of 30; Indiana =10 of 16; Minnesota =2 of 6; Total =40 of 52).

Initial GENELAND analyses conducted to infer the true number of populations produced a clear mode in all analyses (uncertainty coordinates of 1 km, 10 km, 50 km, and 100 km) at $K = 2$. The spatially implicit model performed in GENELAND produced a mode at $K = 2$, in contrast to the ambiguous results in STRUCTURE. The general agreement of GENELAND on the structure of the samples at the four differing uncertainty coordinates (1 km, 10 km, 50 km, 100 km) confirms that uncertainty on spatial coordinates is not a significant parameter in the model (Guillot et al. 2005a).

Figure 1 outlines the results of the GENELAND analysis with K fixed at 2 to determine the probability of population membership for all individuals, given uncertainty coordinates of 1 km, 10 km, 50 km, and 100 km while not considering ambiguity. Red squirrels in Illinois, together with nearby squirrels sampled from Willow Slough Fish and Wildlife Area in Indiana, are grouped into one population, henceforth referred to as the "Illinois" group. All other sampled areas were considered a separate population, hence referred to as the "Midwest" group. All assignments were unambiguous ($Q > 0.7$) except one individual from Pulaski Co., Indiana, which was ambiguously assigned to the Midwest group ($Q = 0.69$).

Analyses performed on the inferred populations based on GENELAND results also indicate a low level of population differentiation. The pair-wise F_{ST} value was 0.03 while the pair-wise F'_{ST} value was 0.22. The Illinois inferred population contained only one unique allele, while the Midwest population contained a high frequency of unique alleles

compared to the state populations (Table 3). The mode shift method performed in BOTTLENECK did not show any evidence of a genetic bottleneck.

Only 2 of the 25 total runs of the MCMC to infer population membership did not group the samples as previously described above (at 1 km uncertainty and 50 km uncertainty). Instead, Michigan (lower peninsula), Indiana, and Illinois clustered as one population while the Minnesota, Wisconsin, and the UP were grouped as another. The pair-wise F_{ST} value reflects the weaker structure when given these two populations (F_{ST} = 0.01; F'_{ST} = 0.13).

DISCUSSION

Origin of the Illinois Red Squirrel Population

The primary objective of this study was to investigate the genetic composition of the small red squirrel population in Illinois to determine its geographic origin. Results from mitochondrial and microsatellite DNA indicate immigration from Indiana and Michigan served as the primary source of the present Illinois red squirrel population. The Illinois-Indiana Φ_{ST} (Table 2), F_{ST}, and F'_{ST} values (Table 4) were the lowest of the respective comparisons and were not significant. Additionally, the low Φ_{ST}, F_{ST}, and F'_{ST} values between Indiana and Michigan reflect genetic similarity between those states, which accounts for the high number of Illinois individuals assigned to Michigan in the traditional assignment test (microsatellite DNA). It has been assumed that westward dispersal of squirrels from Indiana established the extant red squirrel population in Illinois due to geographic proximity. Our results suggest immigration from the northeast (Michigan) combined with dispersal from the east (Indiana), best accounts for the present red squirrel population in Illinois.

Despite the strong evidence for immigration from Indiana, our data also suggest a small number of translocated squirrels from Minnesota may have contributed to the current Illinois population. The individual that shared haplotype F with 11 Minnesota squirrels was sampled at ICSWA, only 2 km west of the Willow Slough State Fish and Wildlife Area in Indiana, but > 600 km from the nearest Minnesota sample. This, in combination with the significant difference between Minnesota and Indiana populations (Tables 2 and 4), indicates haplotype F denotes a true Minnesota lineage, and not an Indiana haplotype that was not sampled. The traditional assignment test also indicates an element of Minnesota ancestry. Consequently, our results suggest a mixed population, comprised primarily of descendents of immigrated individuals from Indiana with a small genetic contribution from Minnesota.

Our conclusions are consistent with a prior study that used skull morphometrics to investigate the origin of the Illinois population (Brown and Edwards 1985). These authors concluded that some Illinois squirrels more closely resembled the Indiana type, whereas others resembled the Minnesota group. The genetic data suggest that Illinois squirrels are most similar to Indiana. We speculate immigration from the source population in Indiana has almost, but not quite, erased the genetic evidence of Minnesota ancestry.

The discovery of red squirrels in northeastern Illinois in the 1970s coincides with a general range expansion of this species in Indiana. Red squirrels were not observed on the Willow Slough Fish and Wildlife Area when it was established in the 1950s but were documented on the site in 1971 (Mumford and Whitaker 1982). By 1977 they were distributed around the area, primarily in pine plantations (Mumford and Whitaker 1982). In this same year, the first red squirrels were confirmed in Illinois along the Kankakee

River near the towns of Momence and Aroma Park, and Kankakee River State Park (Hoffmeister 1989). The precise routes of immigration are unknown, but Mumford and Whitaker (1982) suggested that red squirrels move along riparian corridors in Indiana. Similarly, we found (later in this report) that the current distribution of red squirrels in Illinois is consistent with individuals using forested riparian corridors such as the Kankakee and Iroquois Rivers to disperse. Goheen et al. (2003) suggested the red squirrel has expanded its range in the Midwest at the expense of a competitor, the gray squirrel (*Sciurus carolinensis*). Results of their research indicated that red squirrels were more successful when moving through agricultural fields than gray squirrels, leading them to speculate that red squirrels are less sensitive to fragmentation than gray squirrels.

Regional Patterns of Genetic Variation

The results of the STRUCTURE and GENELAND analyses differ on the estimate of K, the number of genetically discrete populations in the 5-state region. The spatially implicit model of our data in GENELAND indicated two populations, whereas STRUCTURE was ambiguous on the number of populations. All spatially-explicit models in GENELAND (1 km, 10 km, 50 km, 100 km uncertainty) indicated $K = 2$, agreeing with the spatially-implicit model in the same program, offering evidence of substructure that STRUCTURE did not identify. The discrepancy may be attributable to GENELAND initializing K at a uniform distribution for which the user sets K_{min} and K_{max}, making the distribution of K a "soft prior" in the model (Guillot et al. 2005a). The program infers K based on the mode of K along the MCMC. STRUCTURE simply computes the likelihood of the data given a user-defined K. The user then must calculate the most probable K based

on various *ad hoc* methods as discussed previously (Evanno et al. 2005; Pritchard and Wen 2004).

The recent range expansion of red squirrels into Illinois (Hanson 2008) and throughout the lower Midwest may be impacting a competing species, the gray squirrel (*Sciurus carolinensis*; Goheen et al. 2003). Gray squirrels were documented to only inhabit the largest forest patches in a study of mammal communities in Illinois (Rosenblatt et al. 1999) and studies show a long-term decrease in gray squirrel abundance as forest cover decreases (Nixon et al. 1978). Red squirrels and gray squirrels exhibit similar willingness to disperse along fencerows, but the gray squirrel has a higher risk of predation (Goheen et al. 2003).

Our results suggest the red squirrel's ability to traverse unsuitable habitat has resulted in a low degree of genetic differentiation among populations in the Midwest. However, high F_{ST} and F'_{ST} means for the Illinois population differs somewhat from other populations, and the Φ_{ST} pair-wise comparisons confirm this pattern. Although we found no evidence of a founder effect in the Illinois population, the differentiation of the Illinois population is probably attributable to a range expansion into Illinois during the 1970s. Studies have shown that occasional long-distance dispersers may immigrate into an area and establish an isolated, "pocket" population (Ibrahim et al. 1996). Such populations can develop significant genetic differences from the main population. Furthermore, this differentiation may still be observed for hundreds of generations after the main wave of expansion has reached the pocket population (Ibrahim et al. 1996). A similar scenario may have occurred in Illinois and western Indiana and historical records support this claim. Red squirrels were documented in extreme northern Indiana in the late 1800s and early 1900s,

but only recently were documented in Pulaski Co., Indiana (1972), Newton Co., Indiana (1971), and Kankakee Co., (1977; Mumford and Whitaker 1982).

A high level of genetic variation combined with low differentiation has been documented in only a few prior mammalian studies. For example, Simonsen et al. (1998) observed high levels of variation in mtDNA sequences and microsatellites in African buffalo (*Syncerus caffer*), but low levels of differentiation on the regional scale. High levels of differentiation at the local scale were observed in the white-footed mouse (*Peromyscus leucopus*), but there was no evidence of isolation by distance at larger geographic scales (Mossman and Waser 2001). We observed high levels of variability with low levels of differentiation at the regional scale, and no evidence of isolation by distance among red squirrels in the lower Midwest.

The genetic structure of arboreal squirrels has not been well studied in North America. There are limited studies of microsatellite loci in tree squirrels and previous studies of arboreal squirrels in Europe utilized mitochondrial DNA to examine genetic variation in the Eurasian red squirrel (*Sciurus vulgaris*; Finnegan et al. 2007; Barratt et al. 1999). Significant substructure has been documented in the latter species in the Italian Alps using both mtDNA and microsatellite DNA (Trizio et al. 2005). These results are not surprising because the Eurasian red squirrel is considered a habitat specialist, adapted to montane, coniferous forests, and is expected to inhabit more isolated habitat patches with potential for limited gene flow among subpopulations.

In contrast, we did not find significant substructure among Midwestern red squirrel populations. This is also not surprising; previous studies indicate this species persists in deciduous, mixed and coniferous forests, and thus is not a habitat specialist (Goheen and

Swihart 2005; Hanson 2008). Generally, there are few natural geographic barriers or strong environmental gradients that might prompt genetic structure in the Midwest (Stein et al. 2000). Our results suggest that the species' ability to disperse across fragmented landscapes and the region's lack of discrete local environments have combined to produce little genetic variability among red squirrel populations inhabiting this region.

Independent evidence from both mtDNA and microsatellite DNA indicates that the extant population of red squirrels in Illinois is primarily composed of immigrants from Indiana and lower Michigan, but also suggests that a translocation of squirrels from Minnesota contributed to the present population. Microsatellite analyses indicate that the Illinois population may depart from others in the region, not because it includes unique alleles, but rather because it includes the strong genetic signature of immigrants from Indiana combined with an apparent one-time transfer of individuals from Minnesota. This situation has produced a unique admixed population in Illinois, but we found no evidence that these squirrels constitute a genetically-unique remnant population.

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Table 4. The number of haplotypes, haplotype diversity (h), and nucleotide diversity (π) with standard errors in each state for a 272 - 274 bp section of the mtDNA control region in a sample of 229 red squirrels.

State (n)	No. haplotypes	h	π
Illinois (52)	6	0.664 ± 0.045	0.011 ± 0.006
Indiana (50)	13	0.894 ± 0.017	0.023 ± 0.012
Michigan (24) [1]	12	0.917 ± 0.032	0.021 ± 0.012
Minnesota (52)	16	0.838 ± 0.032	0.010 ± 0.006
Wisconsin (51)[2]	17	0.897 ± 0.023	0.016 ± 0.009

[1] Samples from the lower peninsula of Michigan.

[2] Includes 19 samples from the Michigan Upper Peninsula (UP).

Table 5. Lower diagonal: pair-wise comparisons of Φ_{ST} values for a 272 – 274 bp section of the mtDNA control region in a sample of 229 red squirrels from 5 Midwestern states. Mean Φ_{ST} values for each state are listed at the bottom of each column. Upper diagonal: the respective *p* values calculated in ARLEQUIN (v.2.0; Schneider et al. 1997) based on 3000 permutations. Corrections for multiple testing were performed using the false discovery rate method (FDR; Benjamini and Yekutieli 2001). Asterisks indicate comparisons that were significant ($\alpha_{EW} = 0.017$).

	Illinois	Indiana	Michigan	Minnesota	Wisconsin
Illinois	--	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Indiana	0.22*	--	0.200	< 0.001*	< 0.001*
Michigan	0.30*	0.01	--	< 0.001*	0.048
Minnesota	0.62*	0.27*	0.23*	--	< 0.001*
Wisconsin	0.43*	0.11*	0.04	0.12*	--
Means	0.40	0.15	0.15	0.31	0.18

Table 6. Estimates of diversity measures in a sample of red squirrels (N = 232) from the Midwest based on state, and the inferred populations based on GENELAND results. Expected heterozygosity (H_e), observed heterozygosity (H_o), the average number of alleles per locus (A), and the number of unique alleles (A_u) were calculated in the program GDA. The frequency of unique alleles in population is given in parentheses.

	N	H_e	H_o	A	A_u
Given Populations					
Illinois	52	0.76	0.71	8.83	0 (0.0)
Indiana	46	0.79	0.71	10.5	2 (< 0.01)
Michigan	24	0.77	0.69	8.83	3 (0.01)
Minnesota	56	0.78	0.68	11.33	4 (0.01)
Wisconsin	54	0.80	0.68	10.83	3 (0.01)
Inferred Populations					
Illinois	54	0.76	0.72	9	1 (< 0.01)
Midwest	178	0.80	0.69	13.83	30 (0.07)

Table 4. Estimates of F_{ST} (lower diagonal) and F'_{ST} (upper diagonal) for a sample of red squirrels (n = 232) across five Midwestern states. The mean for each state is given below. Significance was calculated with 3000 permutations in the program ARLEQUIN. Corrections for multiple testing were performed using the false discovery rate method (FDR). Asterisks indicate comparisons that were significant (α_{EW} = 0.017).

	Illinois	Indiana	Michigan	Minnesota	Wisconsin
Illinois	-	0.32	0.21	0.27	0.33
Indiana	0.04	-	0.04	0.27	0.14
Michigan	0.03	0.01	-	0.19	0.06
Minnesota	0.04	0.03	0.02	-	0.18
Wisconsin	0.04	0.02	0.01	0.02	-
Mean F_{ST}	0.04	0.03	0.02	0.03	0.02
Mean F'_{ST}	0.28	0.19	0.13	0.23	0.18

Figure 1. Geographic range of *Tamiasciurus hudsonicus* in North America (Obbard 1987).

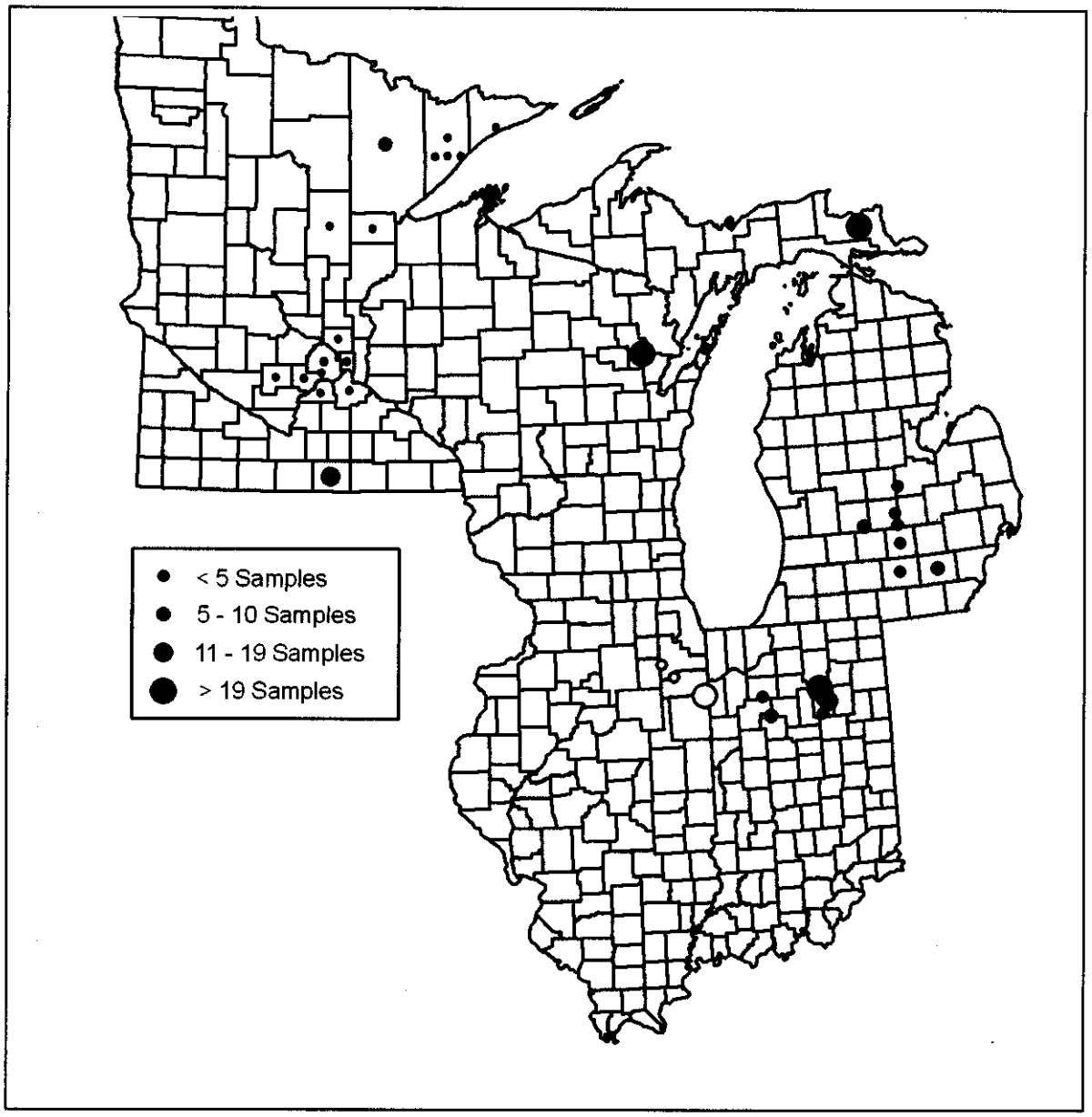

Figure 2. Locations of red squirrel tissue samples in the Midwest used for genetic analyses. Size of the circles reflects the number of samples taken from the locality. Black circles represent one inferred population ("Midwest") while empty circles represent another inferred population ("Illinois") based on the GENELAND results.

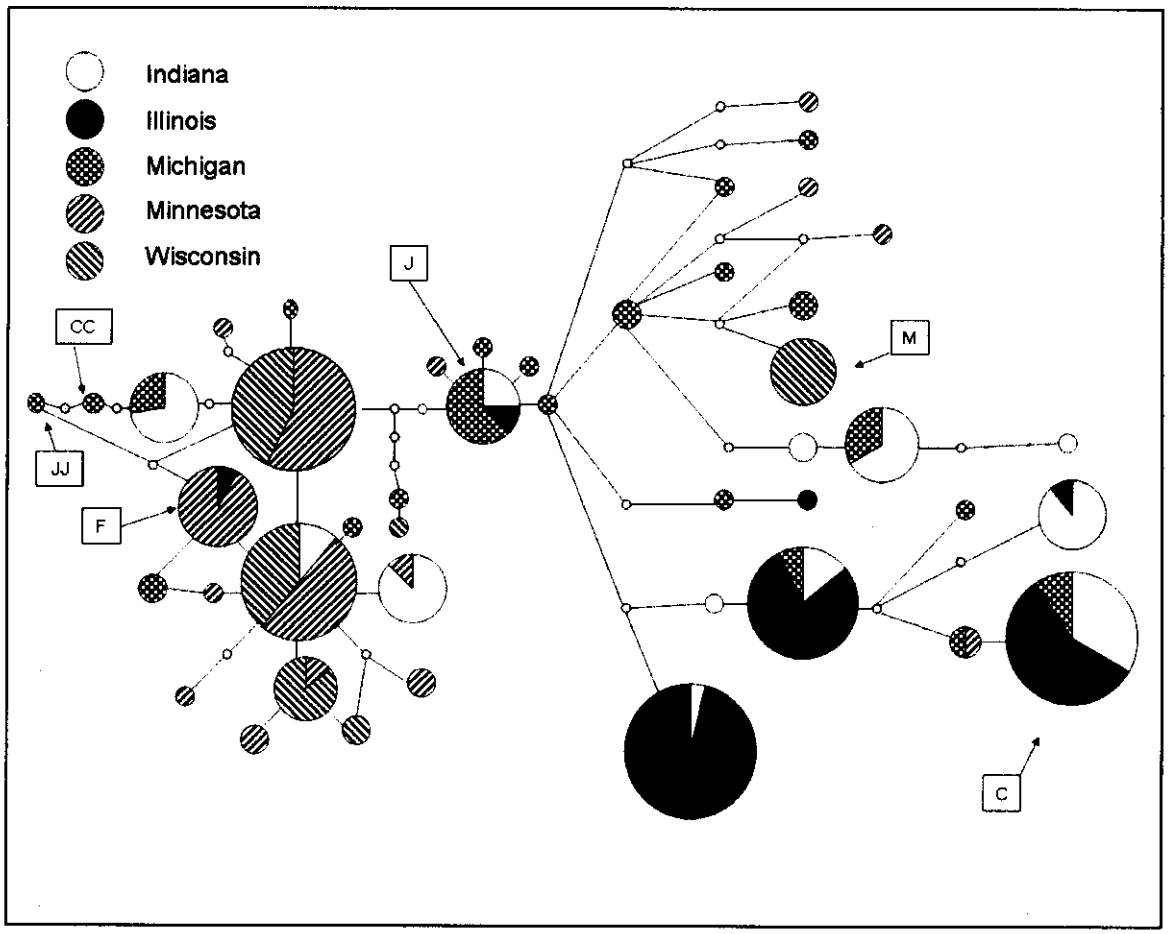

Figure 3. The unrooted haplotype cladogram for a 272 – 274 bp region of the mtDNA control region in a sample of 229 red squirrels. Each circle represents a distinct haplotype and empty circles symbolize intermediate haplotypes not observed in the sample. Haplotypes connected to one another via a line are one mutational step apart. Consequently, haplotypes closer together are more similar. Letters denoting assigned haplotypes are for reference only.

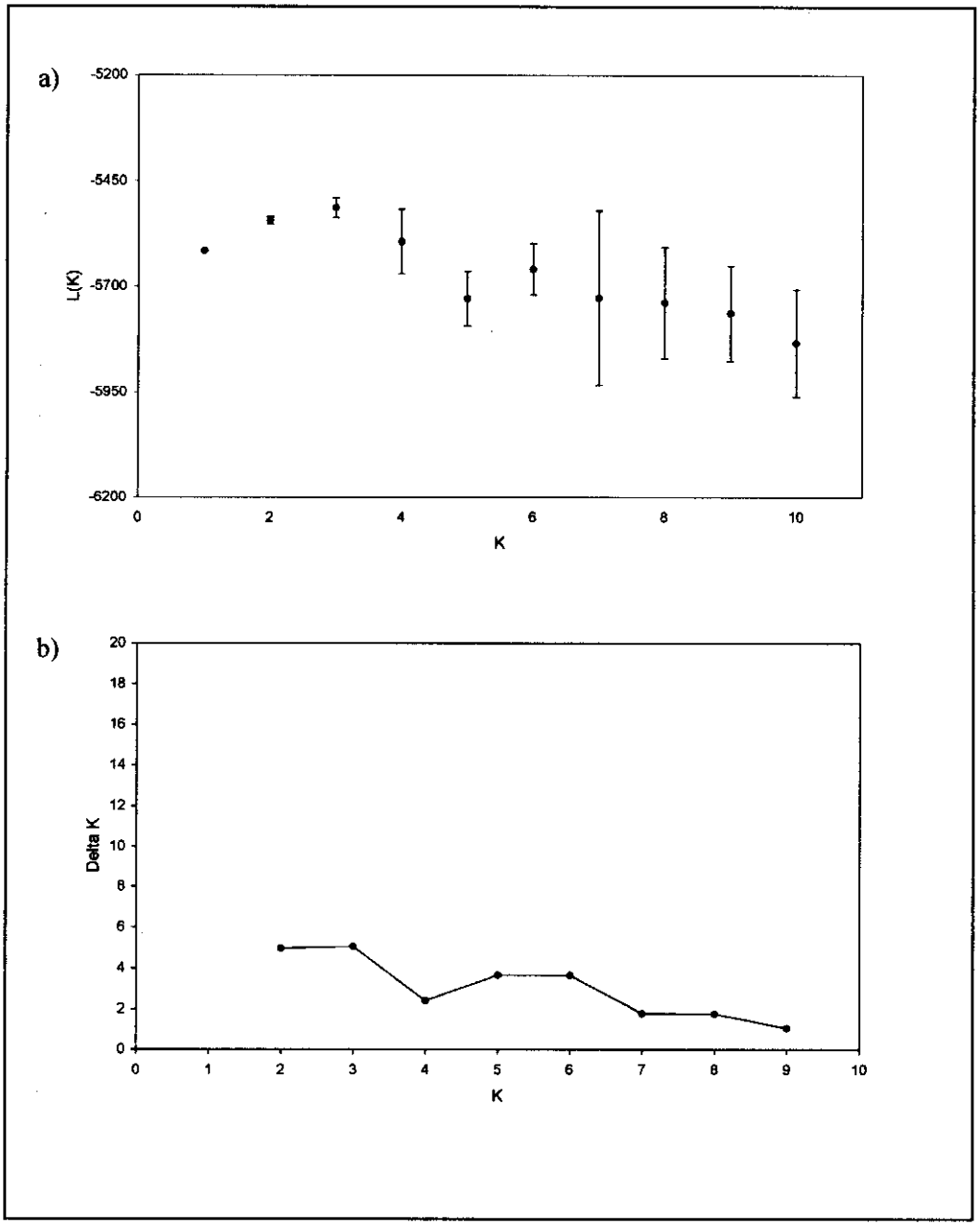

Figure 4. The mean natural logarithm of the probability of the data (a), and ΔK values (b) as calculated based on STRUCTURE results and the method outlined in Evanno et al. (2005).

JOB 2A. DETERMINE THE DISTRIBUTION OF RED SQUIRRELS IN ILLINOIS

INTRODUCTION

Natural history of red squirrels

North American red squirrels (*Tamiasciurus hudsonicus*), commonly referred to as pine squirrels, play an important role in the function of forest ecosystems. These diurnal animals are active year-round and have the ability to impact forest structure and regeneration. By collecting and burying seeds in food caches during preparation for the winter season, red squirrels act as seed dispersers. Smith (1968a) observed that red squirrels may cache up to 16,000 cones of white spruce per year. Red squirrels also serve as important prey for many species, including snakes, raptors, and several mammalian carnivores, notably the marten (*Martes americana*) (Steele 1998). Although red squirrels are primarily granivorous, they are also opportunistic generalists that exploit buds, fungi, shoots, cambium and phloem, nestling songbirds, young gray squirrels (*Sciurus carolinensis*), insect larvae, cottontails (*Sylvilagus floridanus*), beef, and bones (Yahner 2003).

Red squirrels usually are associated with the coniferous forests of Canada, the Rocky Mountains, and the northeastern United States (Figure 1). Optimal habitat includes mature stands of fir (*Abies* and *Pseudotsuga* spp.), spruce (*Picea* spp.), lodgepole pine (*Pinus contorta*), ponderosa pine (*P. ponderosa*), whitebark pine (*P. albicaulis*), and jack pine (*P. banksiana*) that provide ample seeds as forage and closed canopies as shelter and protection from predators (Smith 1968a; Smith 1968b; Rusch and Reeder 1978). Smith (1970) suggested that co-evolution occurs between these squirrels and conifers. Seed predation by the squirrels exerts selective pressure on the trees, resulting in changes in the

reproductive characteristics of the conifers. The pines develop harder cones, which reciprocally selects for squirrels with stronger jaw musculature.

Squirrels inhabiting these coniferous forests have a tendency to larder-hoard pine cones in the western part of their range and scatter-hoard them in the eastern portion (Hurly and Robertson 1990; Dempsey and Keppie 1993). Larder-hoarded conifer cones are stored in a central food cache, or midden, within their territories (Smith 1968a; Kemp and Keith 1970; Rusch and Reeder 1978; Gurnell 1984). Red squirrels in western North America tend to be highly territorial, and aggressively defend their midden from conspecifics and other animals (Yahner 2003). The cones stored in middens are the primary food source for red squirrels during the winter, and territorial behavior occurs in order to secure an adequate year-round food supply (Smith 1968a; Kemp and Keith 1970; Rusch and Reeder 1978; Gurnell 1984).

At the southern extent of their geographic range, red squirrels may inhabit deciduous forests, mixed deciduous-coniferous forests, pine plantations, and fencerows (Smith 1968a). These are thought to be suboptimal habitats, but squirrels can successfully inhabit them as long as a sufficient food supply is present (Yahner 2003). Red squirrels in these regions generally do not display territorial behavior (Obbard 1987).

The colonization of forests in the lower Midwest is thought to be a relatively recent event. In Indiana, the species was not recorded until ~60 years after European settlement (Mumford and Whitaker Jr. 1982). Goheen et al. (2003) suggested that red squirrels may be expanding their range in this region in part due to increasing forest fragmentation caused by agriculture and development. These authors also contended that fragmentation may be contributing to the decline of gray squirrels (*Sciurus carolinensis*) in the Midwest. The

latter species appears to be more sensitive to fragmentation than red squirrels, so that the reduction in gray squirrels may have facilitated the range expansion by red squirrels Goheen et al. (2003).

Red squirrels in Illinois

In the 1800's, red squirrels were present in northern Illinois, however the abundance and distribution of the species at that time remain unclear (Hoffmeister 1989). It is widely believed that red squirrels disappeared from the state in the late 1800's and probably remained absent until the 1970's, when reported sightings occurred in Kankakee and Iroquois counties (Hoffmeister 1989). A likely explanation for this apparent recolonization is that the Kankakee River provides a suitable habitat corridor for the species to immigrate into the state from Indiana.

Another explanation for their apparent reoccurrence in the state in the 1970's is that captive squirrels from Minnesota were translocated and released in eastern Kankakee County during this period. This explanation is firmly held by local residents, but there is no direct evidence of such a translocation.

A third possibility is that the species was never completely extirpated from the state and a remnant population persisted in the Kankakee area (Hoffmeister 1989). Regardless of their origin, red squirrels are currently listed by the Illinois Department of Natural Resources (IDNR) as a "conservation priority mammal" due to their low numbers and restricted geographic range.

Prior to this study, the distribution of red squirrels was thought to be limited to a small area in northeastern Illinois, including parts of Kankakee, Will, and Iroquois counties (Brown 1985; Neely and Heister 1987; Hoffmeister 1989; Figure 5). In this region,

suitable habitat appeared to be restricted to pine plantations composed of red, white, jack, and/or Virginia pines (*Pinus resinosa, P. strobus, P. banksiana, and P. virginiana*, respectively) and small tracts of deciduous or mixed forest embedded in a landscape matrix of corn and soybean fields. We conducted this portion of the study to delineate the current distribution of red squirrels in Illinois and compare past and present ranges to evaluate whether the species' range is expanding, restricting, or stable.

METHODS

Selection of woodlots to be surveyed

We conducted audio-playback surveys to determine the presence of red squirrels in a wide variety of woodlots in northeastern Illinois during the summers of 2006 and 2007. Prior to our first field season, information regarding the known geographic range of red squirrels in Illinois was collected from published records and maps (Hoffmeister 1989) and via interviews with state and regional field biologists at the IDNR, the Illinois Natural History Survey (INHS), and The Nature Conservancy (TNC). These data were used to develop an initial range map showing the species' approximate distribution in the state (Figure 3).

We focused our playback surveys on public lands and forests because time and resources were too limited to survey private parcels extensively. However, when time permitted, private lands were surveyed with permission from the landowners. Forests and woodlots were surveyed in Kankakee, Iroquois, Will, LaSalle, Grundy, Vermilion, and Ogle counties. Potential habitats within the known geographic range of the species were surveyed first, followed by surveys in areas with apparent suitable habitat at the edges of the known range. When squirrels were detected at the edge of the known range, additional

surveys were conducted just beyond this new perimeter in expanding arcs. In turn, when squirrels were not detected at a site, the next habitat patch surveyed was one located closer to the last known woodlot containing the species. By using this approach, the distribution of the red squirrels was systematically investigated in all directions.

Since the geographic range of red squirrels in Illinois extends west from the Illinois/Indiana border, we hypothesized that the species was emigrating westward from Indiana, particularly along the Kankakee River corridor. Therefore, we concentrated our initial surveys in this region. The landscape south and southwest of the known range in Illinois is dominated by row crops and the woodlots here are scarce, small, and isolated. Little public land exists in this area, so the surveys conducted here were restricted primarily to private land. In contrast, the region north of the known range is more urban, encompassing the southern Chicago suburbs. Forest and woodlots here also are fragmented and intensively used for human recreation. To the west, the Kankakee River is bordered by the city of Kankakee. More public land occurs west of the city including Kankakee River State Park (KRSP), the Des Plaines Fish and Wildlife Area (DPFW), Goose Lake Prairie State Natural Area (GLP), and the Chicago-area Forest Preserves.

Conducting Playback surveys

The forests and woodlots constituting potential red squirrel habitat were identified first on digital orthophoto quarter quadrangle (DOQ) aerial photographs using ArcGIS 9.2 (ESRI, Redlands, CA). We used these digitized maps to establish transects parallel to the long axis of each forest patch. Transects were situated 150 m apart, running parallel to each other. Transects were then surveyed using a Global Positioning System (GPS) receiver and compass. Playback stations were situated at 75-m intervals along each

transect and the first and last stations were located ~30 m inside the woodlot edge. We made every effort to ensure complete coverage of the length and breadth of each site.

At each station, we played an audiocassette recording of a red squirrel rattle call (Cornell Ornithology Laboratory, Ithaca, NY) that approximated the volume of a natural call. The call was played for 10-20 s, followed by a 30-s period of listening and watching for squirrel responses. This process was repeated 3 times at each station. Once all stations on all transects had been surveyed, we recorded whether red squirrels were present or apparently absent at each site. Their presence was confirmed only when individuals were seen or heard; however, observations of red squirrel sign (middens or distinctive chew patterns on hard mast) were also recorded. When a squirrel was detected, its location was recorded in UTM coordinates (Zone 16, NAD 83) and any vocalizations and activities of the squirrel were recorded. At these confirmed locations, we recorded the general vegetative composition and structure, including: forest type (deciduous, coniferous, mixed), dominant canopy tree species, relative abundance of shrubs and ground cover, and the presence or absence of walnuts (*Juglans* spp.), pines (*Pinus* spp.), hard mast, and other tree squirrels (*Sciurus niger* and *S. carolinensis*).

Because red squirrels are diurnal and avoid periods of most intense heat in the middle of the day during summer (Obbard 1987), we conducted surveys during the 4-hr periods after sunrise and before sunset during the summers of 2006 and 2007. Additional surveys were conducted during the fall of 2006 and winter of 2006-07. Surveys were not conducted when winds exceeded 20 mph or rain occurred.

Playback surveys were completed at 40 sites in 7 counties in northeastern Illinois. Eighteen sites were visited in 2006 and 22 additional sites were surveyed in 2007. All of

the surveyed woodlots, except for White Pines Forest State Park, were dominated by deciduous hardwoods, particularly oaks (*Quercus* spp.) and hickories (*Carya* spp.). Most of the surveys (38 out of 40) took place in the summer. Three additional surveys were conducted by canoe along the: (1) Kankakee River through Momence Wetlands (Kankakee Co.), (2) Vermilion River through Middle Fork State Fish and Wildlife Area (Vermilion Co.), and (3) Vermilion River through Kickapoo State Park (Vermilion Co.). In these latter surveys, the rattle call was played 3 times at stations placed at 200-m intervals alternating between opposite banks along each river.

Additional sources of information on red squirrel distribution

Additional information on the distribution of red squirrels in Illinois was obtained through phone interviews, posted public notices, and newspaper/newsletter articles. Wildlife biologists throughout northern Illinois provided specific locations of squirrel sightings as well as the dates that squirrels were known to inhabit certain areas. These sources also provided the names and contact information of additional reliable observers and landowners. We developed and placed posters with color photographs of red squirrels and our contact information at parks and wildlife preserves, encouraging the public to contact us if they had any information on red squirrels. Finally, the Kankakee Daily Journal, Illinois Audubon Society, and The Nature Conservancy printed articles about the study with photographs of red squirrels requesting readers to contact us if they observed red squirrels in the region.

RESULTS

Telephone and email surveys

We contacted 18 biologists and talked with many knowledgeable amateurs during this 2-year study. These interviews resulted in 11 new locations for the species, all provided by wildlife professionals in the region. Of these 11, 6 were subsequently confirmed during playback surveys. In addition, one biologist reported that red squirrels were present in Thorn Creek Woods Nature Preserve (near Chicago Heights) in the late 1970's but are now apparently absent. We conducted several surveys in the preserve during the summer of 2007 and found no evidence of red squirrels.

Audio playback surveys

Red squirrels were detected at 11 of 40 sites surveyed, a detection rate of 27.5% (Table 5). The 3 surveys by canoe resulted in no sightings of red squirrels. In each of the 11 sites where red squirrels were detected, the squirrel's response to the recorded call was heard first and then the animal was observed. At only 1 site, a woodlot near Watseka, did we find sign of red squirrels but could not confirm their presence with playback calls. The sign here was discarded walnut shells with chew patterns typical of red squirrels. Based on the age of the material, it appeared that the site had once been occupied, but was not occupied at the time of the survey. We concluded that playback surveys greatly enhanced our likelihood of detecting squirrels in northern Illinois and the method is a practical method for conducting future surveys.

Characteristics of occupied sites

Seven of the 11 woodlots occupied by red squirrels were within 2 km of the Kankakee or Iroquois Rivers. Another site, the Del Rey Department of Transportation site

(DOT), was within 1 km of Spring Creek, an Iroquois River tributary. Two occupied sites (Savicki's private property and Iroquois County State Wildlife Area) were within 10 km of Willow Slough Fish and Wildlife Area in Indiana (WSFWA), an area known to be inhabited by red squirrels. The final occupied site was at Goose Lake Prairie. The woodlot here was small, isolated, surrounded by more than 1,000 ha of prairie and >1.5 km from the nearest woodlot or forested riparian corridor.

Of the 20 surveyed forests and woodlots that were located within 2 km of either the Kankakee, Iroquois, Des Plaines, or Illinois rivers, 7 (35%) were occupied by red squirrels. In contrast, only 4 of 20 (20%) surveyed woodlots that were >2 km from river corridors were occupied. The latter group consisted primarily of isolated woodlots surrounded by corn and soybean fields. Therefore, we suspect that contiguous stretches of riparian forest along these rivers provide habitat and movement corridors for red squirrels.

Red squirrels inhabited primarily deciduous forests, both with and without a component of conifers. The understory of inhabited woodlots varied from sparse to very dense. However, two factors were common to all occupied woodlots: (1) the presence of mature walnut trees, and (2) the absence or scarcity of gray squirrels.

Patch size varied among inhabited woodlots from 5.4 ha to >1,500 ha. In addition, the shape of inhabited woodlots varied from narrow, linear patches to wide, contiguous forests. We found no evidence that the size or shape of patches influenced occupancy. Several woodlots that were <20 ha in size were occupied, suggesting that this species is not particularly area sensitive. In addition, although most occupied patches were <2 km from forested riparian corridors, several were >2 km from the nearest apparent habitat. This suggests that these squirrels are capable of dispersing across relatively wide stretches of

non-forested landscape. For example, squirrels were found on both sides of major rivers in the region, on both sides of Interstates 55 and 57, east and west of Kankakee and its suburbs, and in at least one woodlot surrounded by 1,000 ha of restored prairie.

Current geographic range

The results from playback surveys and personal interviews provided the necessary data to construct a current geographic range map for red squirrels in Illinois (Figure 6). Our results suggest that the species is limited to a portion of northeastern Illinois south of Chicago, but their geographic range is broader than previously reported. Our surveys extend the species' range by 50 km to the southwest and 30 km to the northwest. In addition, the northern extent of the range now appears to be 20 km further south than was previously reported. Whether this range contraction is related to urban expansion of the south Chicago suburbs cannot be confirmed, but seems likely.

In summary, our research suggests that red squirrels occupy both isolated woodlots and contiguous riparian forested corridors in at least 4 counties in northeastern Illinois. Occupied habitat included deciduous forests, mixed deciduous-pine forests, and pine plantations across a broader area of northeastern Illinois than previously known. We cannot say however whether this apparent range expansion is due to successful emigration by the squirrels or more effective and extensive surveys than have previously been conducted.

DISCUSSION

The use of playback surveys resulted in the sightings of red squirrels at 11 different sites across northeastern Illinois. The detection of red squirrels at 3 of these sites (DPFW, GLP, and DOT woodlots) illustrates how the outer perimeter of the geographic range has

apparently expanded to the northwest and southwest. The presence of red squirrels at these locations had not been documented prior to this study.

Telephone and email surveys of wildlife biologists provided additional useful information regarding new sites occupied by squirrels, as well as an approximate timeframe for occupation at several sites. Although many reports were for sites within the previously-known geographic range, 3 reports extended the known range to include: Goose Lake Prairie (GLP), a woodlot near Clifton High School (CHS) in Iroquois County, and a woodlot in Custer Park, near Wilmington in Will County. The report of squirrels at GLP resulted in a subsequent playback survey at that site that confirmed the presence of red squirrels. The reports for CHS and CP were beyond the previously-known range and eventually served to fill spatial gaps between more distant occupied sites and occupied sites closer to the center of the range.

These new locations, in conjunction with temporal information provided by wildlife professionals, suggest that red squirrels have expanded their range in Illinois during the past 30 years, primarily to the west and south. In turn, there has been an apparent contraction at the northern extent of the range, south of Chicago Heights and the south suburbs. It seems unlikely that squirrels have been present at these new locations, but gone unnoticed for the past 30 years. Sites such as Goose Lake Prairie and Des Plaines Fish and Wildlife Area are intensively managed and frequently visited by outdoor enthusiasts. If red squirrels had occupied these sites for long, they likely would have been detected and reported.

We hypothesize that red squirrels are emigrating to the northwest along the Kankakee River corridor and southwest along the Iroquois River corridor and its tributaries

(Figure 6). Forested corridors along these rivers provide suitable habitat and facilitate movement and range expansion. This appears to be coincidental with Goheen et al.'s (2003) observations that red squirrels are expanding their range in Indiana .

Our results also suggest that range contraction may have occurred south of the Chicago metropolitan area. For example, red squirrels were present at Thorn Creek Woods Nature Preserve (TCW) in the late 1970's, but intensive playback surveys and field observations at that site and surrounding habitats produced no evidence of squirrels. Obviously, a number of factors could lead cause squirrels to abandon this area, but we speculate that increased urbanization and roadway development may have contributed to this range contraction.

Although squirrels were found predominantly in forests and woodlots on or near river corridors, the animals also were found in small, isolated woodlots surrounded by grasslands and agricultural fields. The GLP site was the most notable example of this. A red squirrel was detected in a small (5 ha), deciduous woodlot that is surrounded by restored prairie and actively managed with prescribed burns. Perceived predation risk and energetic costs tend to be higher in risky habitats such as forest clear-cuts (Bakker and Van Vuren 2004). In Illinois, grasslands and cropfields appear to be risky habitats for red squirrels. Our results are consistent with those of others who have suggested that these vegetation types are not insurmountable barriers to the movement of red squirrels and that this species is relatively insensitive to the isolation of woodlots (Nupp and Swihart 2000; Goheen et al. 2003).

We successfully detected red squirrels in 11 of 40 surveyed forests across northeastern Illinois; however, we were unable to prove definitively that squirrels were

absent from the other 29 surveyed. An important apparent trend that emerged during the playback surveys was that gray squirrels were more common in woodlots lacking red squirrels than in those occupied by red squirrels. We cannot say whether this represents a causal effect of competition between these species or differences in habitat preferences. However, gray and red squirrels share many resources and a high degree of interspecific competition can occur between them (Nupp and Swihart 2001). Our observations are consistent with Goheen et al.'s (2003) notion that the persistence of red squirrels in Midwestern deciduous woodlots may be facilitated by the decline in gray squirrels caused by forest fragmentation. Our observations suggest that the abundance of red squirrels and gray squirrels are inversely correlated in the deciduous forests where the two species are sympatric. Our study was not designed to test this hypothesis, but it merits future research.

During playback surveys, all detected red squirrels vocalized in response to the audio-recordings. Red squirrels have five calls: the rattle, screech, chirp, buzz, and growl (Smith 1968; Smith 1978; Lair 1990; Greene and Meagher 1998). Each serves a particular purpose and facilitates specific behavioral responses from conspecifics and other species. The rattle, screech, buzz, and growl calls are associated with territorial and aggressive behavior aimed at conspecifics. In contrast, the chirp is as an alarm call used in the presence of potential predatory threats (Greene and Meagher 1998). We used the rattle call during playback surveys and most individuals responded by rattling back. Therefore, red squirrels in Illinois do respond to this territorial call, even though the species does not appear to be territorial in this region and we found little evidence of territoriality in our study area. The rattle call may have a more general purpose in this region, possibly notifying other squirrels of the caller's presence.

In conclusion, we delineated the current geographic range of red squirrels in northern Illinois. The presence and apparent absence of the squirrels was documented in individual forests using playback surveys and personal interviews with biologists and knowledgeable individuals in the region. These results suggest that red squirrels have likely expanded their range to the west during the past 30 years, apparently using the riparian forests in the Kankakee and Iroquois River watersheds as primary movement corridors. Grasslands, agricultural fields, and highways may slow dispersal, but they have not blocked range expansion.

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Table 5. Results and locations of playback surveys in northeastern Illinois.

#	Site surveyed	County	Squirrels present
1	Goose Lake Prairie State Natural Area	Grundy	X
2	Gebhart Woods & section of I&M Canal	Grundy	
3	Iroquois Co. State Wildlife Area	Iroquois	X
4	Hooper Branch Savanna	Iroquois	
5	Del Rey DOT site	Iroquois	X
6	Savickis private property	Iroquois	X
7	Bronson private property	Iroquois	X
8	Specchio private property (near Loda)	Iroquois	
9	Private land near Loda	Iroquois	
10	Private property near Woodland	Iroquois	
11	State-owned property (old Girl Scout camp)	Iroquois	
12	Iroquois Woods	Kankakee	X
13	Kankakee River State Park selected woodlots	Kankakee	X
14	TNC property: Pembroke township	Kankakee	
15	Island Park, Momence	Kankakee	X
16	Sweet Fern private property	Kankakee	
17	Aroma Park Forest Preserve	Kankakee	
18	Momence Wetlands selected woodlots	Kankakee	
19	TNC property: Bentley/Crawford	Kankakee	
20	TNC property: Tallmadge	Kankakee	
21	Waldon Arboretum	Kankakee	X
22	TNC property	Kankakee	
23	Gooseberry Island Nature Preserve	Kankakee	
24	Bourbonnais Geological Area	Kankakee	X
25	Island in Kankakee River State Park	Kankakee	
26	Illini State Park	LaSalle	
27	Starved Rock State Park	LaSalle	
28	Matthiessen State Park	LaSalle	
29	White Pines Forest State Park	Ogle	
30	Middle Fork Wildlife Area selected woodlot	Vermilion	
31	Kickapoo State Park selected woodlot	Vermilion	
32	Raccoon Grove Forest Preserve	Will	
33	Des Plaines Fish and Wildlife Area	Will	X
34	Messenger Woods Nature Preserve	Will	
35	Hitts Siding Nature Preserve	Will	
36	Laughton Preserve	Will	
37	Thorn Creek Woods Nature Preserve	Will	
38	Hickory Creek Preserve	Will	
39	Hammel Woods	Will	
40	Rock Run Preserve	Will	

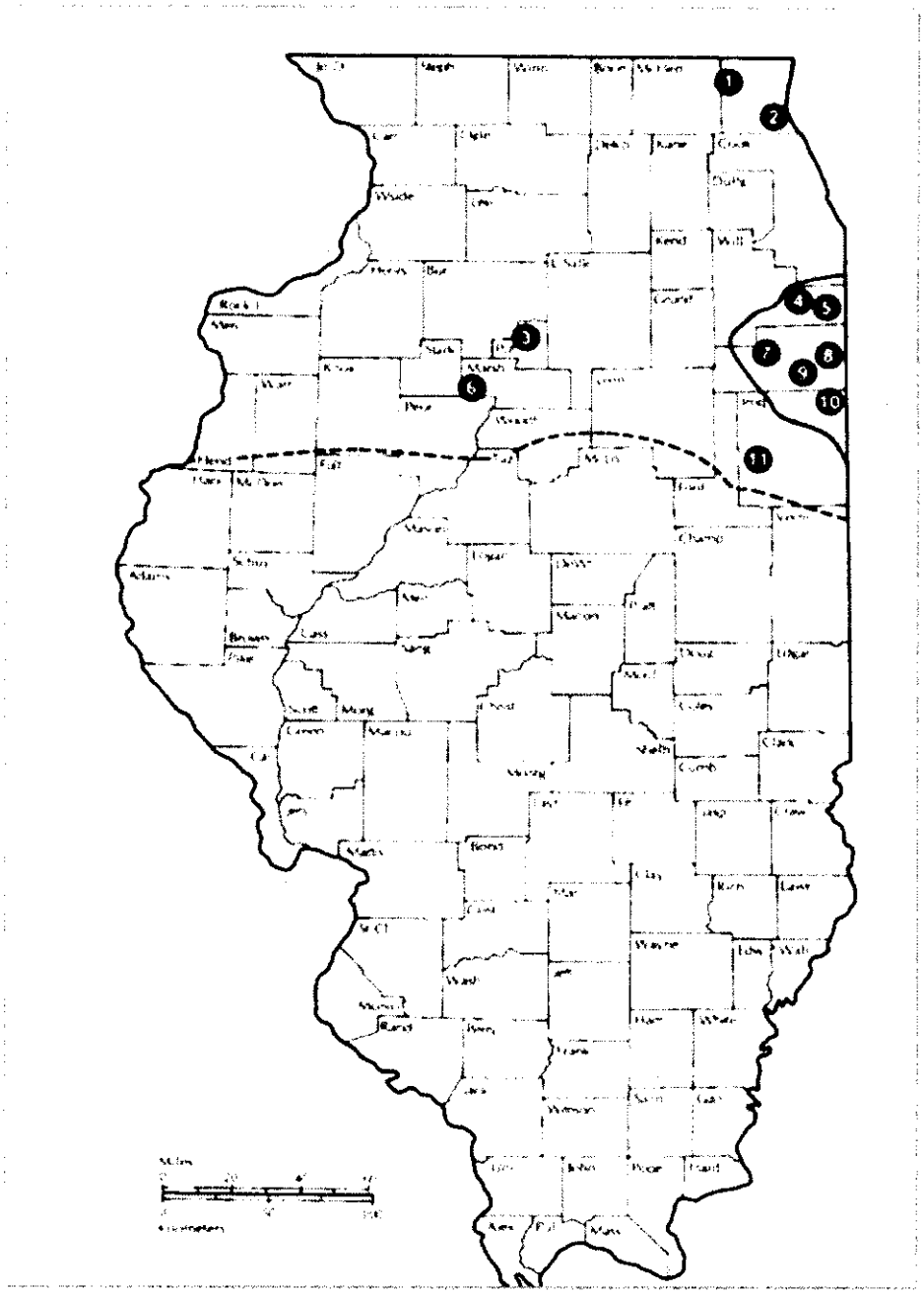

Figure 5. Presumed range of red squirrels in Illinois prior to this study (Wilson and Ruff 1999). The historic range is the area north of the dotted line. The 1980's range is the area within the solid line. Numbered points indicate documented records of occurrence.

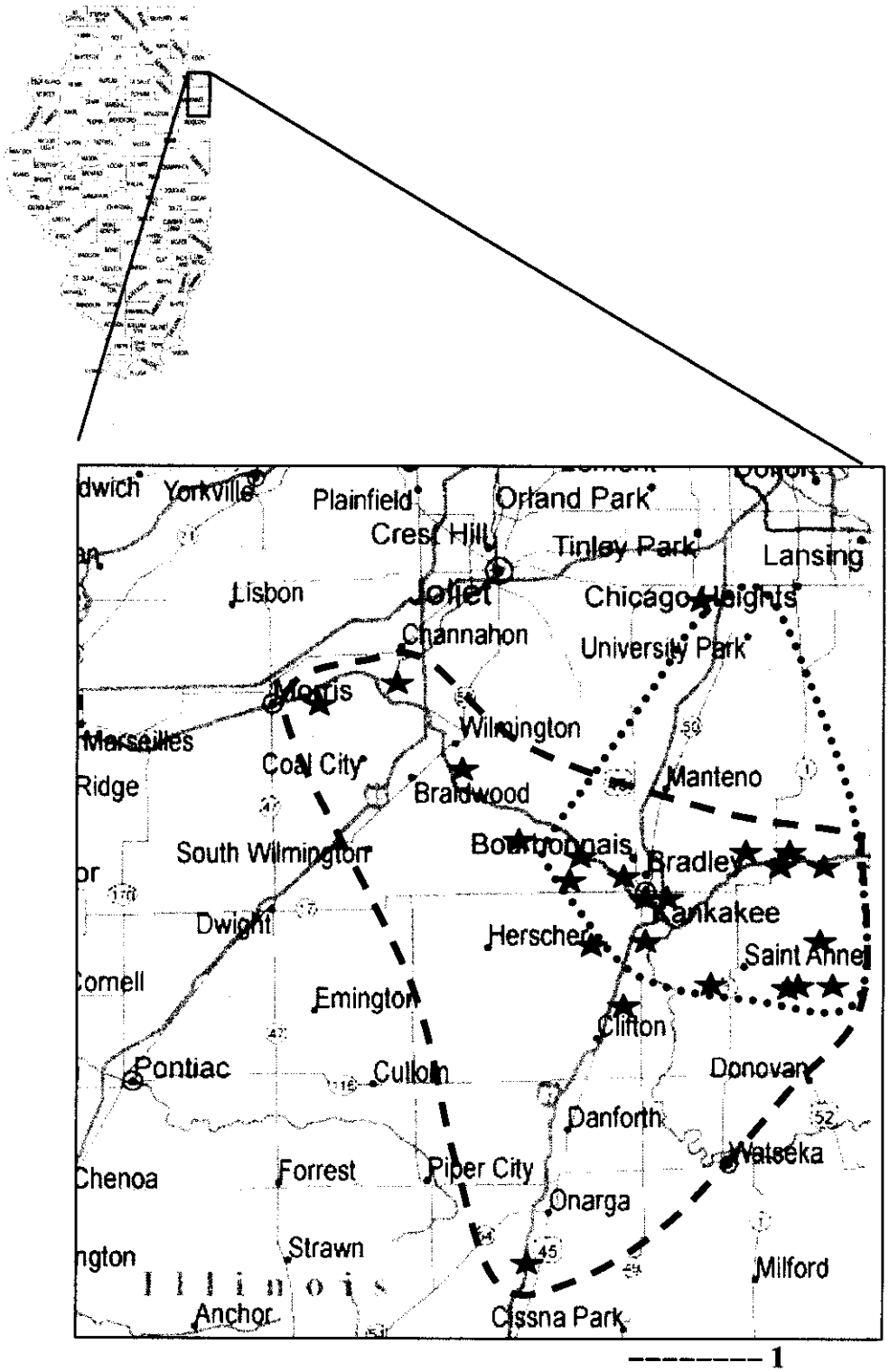

Figure 6. Geographic range maps showing the known range of red squirrels in Illinois before and after our study. The dotted line indicates the known range prior to our study; the dashed line indicates the known range after our study. Stars indicate locations currently inhabited by red squirrels.

JOB 2B. INVESTIGATE HABITAT USE AND LIMITING FACTORS OF RED SQUIRRELS IN ILLINOIS

INTRODUCTION

In the fragmented, agricultural landscapes of the lower Midwest, red squirrels often occupy deciduous and mixed deciduous-coniferous woodlots. For example, in northern Ohio and northern and central Indiana, they occupy deciduous and mixed forests and pine plantations (M. Reynolds, Ohio DNR, pers. comm.; Goheen and Swihart 2003). These forests are thought to be suboptimal habitats relative to native coniferous forests; however, red squirrel populations can remain viable in deciduous and mixed forests provided that adequate food resources are available (Yahner 2003).

Although red squirrel habitat in the lower Midwest may be suboptimal, few studies have addressed or quantified habitat use by red squirrels in this region. Research in Indiana suggested that the presence of red squirrels in deciduous woodlots is positively correlated with the presence of black walnuts (*Juglans nigra*) and the squirrels show a preference for stands containing this species (Goheen and Swihart 2005). Another Indiana study suggested that the squirrels prefer deciduous woodlots with at least some coniferous component (Ivan and Swihart 2000). However, Goheen and Swihart (2005) reported that red squirrels in Indiana showed no preference for pine plantations, contrary to what might be expected of a species so well-adapted to coniferous forests. However, it is important to note that pine plantations differ from natural forests in structure, primary production, and biodiversity.

Although red squirrels inhabiting northern coniferous forests are highly territorial, the home ranges of red squirrels occupying deciduous forests often overlap broadly (Layne

1954; Smith 1968a; Rusch and Reeder 1978). Previous studies have shown that the size of these home ranges may be influenced by habitat type, location, and the method of calculation, but general sizes usually vary from ~0.3-1.5 ha (Yahner 2003). Twenty years ago, Wright (1985) initiated a study to quantify home range size, overlap, and territorial behavior by red squirrels in northeastern Illinois. However, his sample sizes were small and few conclusions regarding habitat preferences and home range size could be derived.

Since 2000, the IDNR has been actively girdling and removing non-native pines from public lands in northeastern Illinois. The potential effect of this program on the resident red squirrel population had not been addressed prior to our study and there was concern that the removal of pines might eliminate the last vestiges of suitable habitat for red squirrels in the region. Therefore, we conducted this portion of the study to quantify home range size, habitat use, and habitat preferences of red squirrels in northeastern Illinois. The primary purposes of this research were to contribute to the conservation and management of red squirrels in Illinois by identifying: (1) critical habitats needed for the species to persist in the state, and (2) minimum areas of habitat needed for individuals to meet their resource needs. This information is necessary for develop scientifically-sound recommendations for habitat management, particularly regarding the potential impact of pine removal on this species of conservation concern.

METHODS

Study site

This portion of the study was conducted at Iroquois County State Wildlife Area (ICSWA) in northeastern Illinois during the summers of 2006 and 2007. ICSWA is located at the northeast corner of Iroquois County (40.993374 latitude, -87.598457 longitude)

(Figure 4). The area encompasses 1,004 ha comprised of deciduous forests, pine plantations, marshes, prairies, and oak savannas. The site was selected because it is one of the few publicly-owned sites in Illinois inhabited by a relatively large red squirrel population. ICSWA also contains a variety of habitat types, including deciduous and mixed forests, pine plantations, old fields in various stages of succession, and grasslands. Therefore, squirrels occupying the site apparently had "choices" among these habitat types in which to establish their home ranges and seek necessary life requisites.

Trapping and radiotelemetry

From mid-May through August each year, live traps (40 x 12.6 x 12.6 cm; model 603, Tomahawk Live Trap Co., Tomahawk, WI) were placed in grids covering each of five habitat types in proportion to their occurrence on the study site. Traps were baited with sunflower seeds and corn, peanut butter and oats, or shelled walnuts. We opened traps at dawn each day and closed them at dusk to reduce the capture of non-target nocturnal animals. Trapping was suspended during periods of inclement weather. During the day, traps were checked every 3-4 hrs to prevent unnecessary trauma to captured animals. Trapped squirrels were transferred from the trap into a cloth handling cone (Koprowski 2002) where they were anesthetized with an intramuscular injection of 0.02 mg/gm ketamine plus 0.001 mg/gm xylazine (Kreeger et al. 2002). Once anesthetized, selected individuals were outfitted with a nylon-coated radiocollar with a whip antenna (2.5-4 g; PD-2C, Holohil Systems Ltd., Ontario, Canada). These collars weigh $\leq$ 2.3% of an adult red squirrel's body mass. Numbered ear tags (model 105-3; National Band and Tag Co., Newport, KY) were applied to the right ear, body mass was measured with a spring scale

(Pesola AG, Barr, Switzerland) accurate to 1 g, and total body length was measured with a metric tape measure.

Tissue samples were collected for subsequent genetic analyses with a 2mm biopsy punch from the pinna of the left ear, following the methods of Trizio et al. (2005). These samples were then stored in dessicant vials for DNA analysis. Trapping and handling protocols were approved by Eastern Illinois University's Animal Care and Use Committee under permit EIU06010 and followed guidelines set forth by the American Society of Mammalogists (Animal Care and Use Committee 1998).

Individual red squirrels were radiolocated throughout both summers at least once daily. Squirrels were located by homing on the signal or triangulating at close range (~30 m) using a directional yagi antenna (Mech 1983). Homing was most common, allowing us to detect and record the animal's specific location, as well as the habitat type, dominant canopy trees, and understory vegetation at each location. When triangulation was necessary, 3-4 bearings were recorded at ~70-110° angles from 20-30 m away from the individual. Controlled tests conducted on transmitters placed in known locations resulted in a mean angular error of 2 m (SE = 0.3). Each location was identified using a GPS receiver (Garmin GPS III+, Olathe, KS) in UTM coordinates (NAD 83, Zone 16). We used ArcGIS (version 9.2) to overlay all radiolocations onto current 1.5-m resolution digital orthophoto quarter quadrangle (DOQ) aerial photographs (Illinois Natural Resources Geospatial Data Clearinghouse 2006) for analyses. We conducted telemetry at different times throughout the day in order to capture the full range of each squirrel's daily movements.

It is typical for an animal's movements to be autocorrelated, as individuals rarely move in a completely random manner. Therefore, in our study, we tried to reduce temporal autocorrelation by ensuring that the minimum time-interval between successive radiolocations was 2 hrs. This interval was reasonable because it exceeded the time necessary for squirrels to traverse their home ranges. It also increased the likelihood that radiolocations were biologically independent, thus reducing temporal autocorrelation between locations (Lair 1987). Several researchers have suggested that autocorrelated locations should be completely eliminated before home ranges are estimated (Swihart and Slade 1985; Worton 1987; Ackerman et al. 1990; Kenward 1992). Whereas complete elimination facilitates statistical significance, this also reduces sample sizes and can diminish the researchers' ability to discern biologically-significant patterns of habitat use (deSolla et al. 1999). Furthermore, complete elimination of autocorrelation usually reduces statistical power and the accuracy of home range estimates and can cause the potential loss of biologically important information (deSolla et al. 1999). Therefore, we made the a priori decision to include all locations for a squirrel separated temporally by at least 2 hrs.

Home range estimation

Home ranges were calculated for individuals with ≥30 locations using Home Range Tools for ArcGIS (Rodgers et al. 2005) to optimize the accuracy of home range estimates (Seaman et al. 1999). Two types of home ranges were calculated, the 90% fixed kernel (hereafter referred to as the home range) and the 50% fixed kernel (hereafter termed the core area; Figure 5). We elected to not calculate the minimum convex polygon (MCP) home range due to its numerous limitations (Jennrich and Turner 1969; Worton 1987; Harris et al. 1990; White and Garrott 1990). In addition, the MCP estimates are not useful

for comparisons to previous research because of their sensitivity to sample size (Seaman et al. 1999). Alternatively, kernel estimators are less sensitive to sample size and provide a more accurate estimate of home range size. Kernel estimates are nonparametric probability density estimators of the utilization distribution. The use of kernel methods to estimate home ranges is increasingly favored over other parametric methods (Worton 1989). The 90% kernel range considers areas that are usually used and excludes outlying locations that are rarely used. In turn, the 50% kernel core area shows the individual's areas of heaviest use in which the animal was located most often.

When using kernel estimates, it is important to select an appropriate smoothing parameter, or bandwidth (h). Several different automated methods exist for generating appropriate bandwidths including: (1) HREF, (2) least-squares cross-validation (LSCV) (Worton 1989), and (3) biased cross-validation (BCV). We decided against using HREF because of its tendency to over-smooth the utility distribution (Rodgers and Carr 1998). The method is effective when animals have unimodal utility distributions (Worton 1995), but many of the squirrels had multiple centers of activity within their home ranges. The BCV method differs from the LSCV method in that it finds an h-value in which the asymptotic mean integrated square error is minimized, rather than the mean integrated square error (Sain et al. 1994). Sain et al. (1994) showed that BCV can perform well in simulation studies, but when we tested the method with our data, utilization distributions tended to be over-smoothed. After further investigation, the bandwidth resulting from the LSCV method (Silverman 1986) appeared to be most suitable for our data. Out of the three automated methods described above, the kernels derived from this method appeared to most accurately capture the animals' home ranges.

Quantifying habitat use

Based on the predominant vegetation on the study site, five habitat types were identified: deciduous forests, pine plantations, brush-savannas, grasslands, and cropfields. Dominant tree genera within deciduous forests were oaks (*Quercus* spp.) and black cherry (*Prunus serotina*). Pine plantations were composed primarily of scotch pine (*Pinus sylvestris*), but red and white pines were also present. The brush-savanna category included several small patches that were distinctive in composition with open canopies containing sparse pines and hardwoods, and extremely dense understories dominated by blackberries (*Rubus* spp). Downed trees and woody debris also were present in this habitat type. Grasslands included marshes, wet and dry prairies, and sand dune vegetation. Cropfields were planted to either corn or soybeans. These five habitat types were digitized on a 2005 DOQ basemap using ArcMap 9.2, (ESRI, Redlands, CA).

To investigate habitat use and preference, the entire 1,004-ha ICSWA and 751 ha of surrounding private land were classified as available habitat for radiocollared squirrels. We then overlayed each squirrel's individual radiolocations, core area contours, and home range contours on the basemap. Habitat use then was investigated at 2 spatial scales: (1) habitat selection at the scale of the home range (comparing habitat composition of home ranges to that of the study area), and (2) selection at the scale of the core area (comparing the composition of the core area to that of the home range). These scales are similar to Johnson's (1980) definition of 2nd-order habitat selection (habitat composition within home ranges versus that of the geographic range) and 3rd-order selection (composition of heavily used areas versus that of the home range), respectively.

Data analysis

Because various resource selection methods can yield different results and disagreement in the literature exists as to what method should be preferred (Alldredge and Ratti 1986; Alldredge and Ratti 1992; McClean et al. 1998; Manly et al. 2002), we evaluated habitat use and preferences using two common methods: (1) chi-square analysis (Neu et al. 1974), and (2) compositional analysis (Aebischer et al. 1993).

Neu et al. (1974) introduced the use of a X^2 test to determine relative preference or avoidance of specific habitat types. The null hypothesis is that habitat types will be used in proportion to their availability. If the overall test is significant, individual preferred and avoided habitats can be evaluated with Bonferroni confidence intervals around the proportions. Cherry (1996) suggested that Bailey confidence intervals (Bailey 1980) with a continuity correction should be used instead of the Bonferroni confidence intervals utilized in Neu et al. (1974). Therefore, we used 95% Bailey confidence intervals around the proportions for this analysis.

To determine how different habitat types ranked in relative preference, we also conducted a compositional analysis (Aebischer et al. 1993). This method uses MANOVA techniques to examine two data sets (used versus available habitats) which are expressed as proportions. We used Leban's resource selection software (1999) to investigate second- and third-order resource selection using both the X^2 test and compositional analysis. The compositional analysis was performed in SAS (version 9.1) using the program BYCOMP.SAS (Ott and Hovey 1997) to verify results.

Resource selection studies examine the ways that animals use their available habitat. Three potential sampling designs were identified by Thomas and Taylor (1990):

(1) population-level resource selection is assessed by examining availability and use of habitat by all animals in a sample; individuals are not recognized, (2) habitat use is assessed using individual animals and then use is pooled across all individuals, and (3) habitat use and availability are assessed separately for each animal. We used the second design because habitat selection is examined for individual animals, all data are used, individual variation can be considered, and our goal was to extract general patterns of habitat use for our study population (Braun 2005).

Finally, we used ANOVA to test whether canopy cover varied between used and unused sites. Canopy cover was measured with a densiometer at individual red squirrel locations and at 110 random sites within the study area for comparison.

RESULTS

Trapping and marking

A total of 103 red squirrels was captured, including 41 individual squirrels and 62 recaptures. We trapped for 3,660 trap-days in 2006 and 1,780 trap-days in 2007; therefore, our overall success rate was 52.8 trap-days per red squirrel captured. Three other sciurids inhabit the study area: gray squirrels, fox squirrels (*Sciurus niger*), and eastern chipmunks (*Tamias striatus*). During the 2 summers, we trapped only 1 gray squirrel and 8 fox squirrels, for a rate of 604.4 trap-days per tree squirrel captured. In contrast, eastern chipmunks (*Tamias striatus*) were very abundant on the study area and we caught 711 of these, resulting in a capture rate of 7.7 trap-days per chipmunk.

The physical condition of squirrels did not appear to be impacted by handling or the radiocollars. Three animals collared in 2006 were recaptured in 2007. Two of these showed no evident ill-effects from wearing the collars for >12 months; the third was

missing some hair under the collar apparently from wear, but no skin damage or infection had occurred. The wounds from biopsy punches were healed in all 3 squirrels and none showed any signs of injury or infection around metal ear tags. Similarly, squirrels recaptured within days or a few weeks of their initial capture showed no signs of physical impairment or infection associated with their collars, ear tags, or biopsy punches. Red squirrels appeared to recover quickly from handling and invasive procedures.

Home range size and use of core areas

Thirty-nine individuals (18 females, 21 males) fitted with radiocollars were tracked to investigate their movements and habitat use. One female was predated after only 5 days, so she was eliminated from subsequent home range analyses. In addition, 3 males tracked in 2006 were recaptured, fitted with new collars, and tracked again in 2007. After analyzing radiolocations for each summer, their home ranges were deemed to be independent between years and we treated each home range separately in subsequent calculations. Collectively, we calculated 41 home ranges and core areas for 38 individual squirrels based on 1,554 individual radiolocations, a mean of 38 (SE = 0.79) locations per squirrel.

The mean home range size for these individuals was 1.7 ha (SE = 0.24). Core areas averaged 0.4 ha (SE = 0.06). Wilcoxon rank-sum tests revealed no differences in home range sizes between males (x =1.8 ha; SE = 0.38 ha) and females (x = 1.5; SE = 0.31 ha) (U = 340, df = 40, P = 0.66). In addition, no differences were found in the size of core areas used by males (x = 0.49, SE = 0.11 ha) versus females (x = 0.37, SE = 0.08 ha) (U = 340, df = 40, P = 0.66). Home range sizes also did not differ between adults (x = 1.8, SE = 0.41 ha) and juveniles (x = 1.2, SE = 0.24 ha) (U = 144, df = 40, P = 0.17). Nor did core area

sizes differ between adults (x = 0.47, SE = 0.10) and juveniles (x = 0.35, SE = 0.07) (U = 146, df = 40, P = 0.19). The sizes of home ranges did not differ between 2006 (x = 1.8, SE = 0.36) and 2007 (x = 1.6, SE = 0.34) (U = 347, df = 40, P = 0.80), nor did core area sizes differ between 2006 (x = 0.47, SE = 0.10) and 2007 (x = 0.39, SE = 0.09) (U = 346, df = 40, P = 0.78). Because the sizes of home ranges and core areas did not differ in among sexes, ages or years, we pooled these data for subsequent analyses of habitat use and preference. The home ranges and core areas of squirrels frequently overlapped among individuals, suggesting that these squirrels are not territorial in summer and fall. Figure 6 illustrates overlapping core areas among individual squirrels in 2006 and 2007, respectively.

Habitat use and preference

X^2 tests showed that the habitat composition of home ranges differed significantly from that of the study area, suggesting that 2nd-order habitat use is selective (G_{adj} = 7670, P < 0.0001). Therefore, Bailey simultaneous 95% confidence intervals were calculated to investigate which habitat types were preferred and avoided. Results showed that grasslands and cropfields were avoided (X^2 = 670, df = 4, P < 0.0001 and X^2 = 940, df = 4, P < 0.0001, respectively; Table 2). In contrast, all 3 wooded habitats were preferentially selected (pines: X^2 = 3238, df = 4, P < 0.0001; deciduous forest: X^2 = 210, df = 4, P < 0.0001; brush savanna: X^2 = 42,296, df = 4, P < 0.0001), as would be expected for a tree squirrel.

When we then compared the habitat composition of core areas to that of the home ranges, 3rd-order habitat selection also was evident (G_{adj} = 281, P < 0.0001). Squirrels generally avoided grasslands (X^2 = 80, df = 4, P < 0.0001) and cropfields (X^2 = 49, df = 4,

$P < 0.0001$) within their home ranges, instead utilizing core areas dominated by pines ($X^2 = 16$, $df = 4$, $P < 0.05$) and brush-savanna ($X^2 = 98$, $df = 4$, $P < 0.0001$; Table 3). However, in contrast to the 2nd-order selection, these results further suggest that squirrels tended to avoid deciduous stands ($X^2 = 10$, $df = 4$, $P < 0.05$) within their home ranges, if pines or brush-savanna habitats were available.

To further evaluate the relative use of habitat types, particularly the forest classes, by red squirrels at ICSWA, we next used compositional analysis to rank preferred habitat types at the 2nd- and 3rd-order levels of habitat selection (Tables 4 and 5, respectively). These results allowed us to reject the null hypothesis that the composition of the red squirrels' home ranges was proportional to that of the study area (Wilk's $\lambda = 0.17$, $P < 0.0001$). Using this technique, habitat preferences at the 2nd-order level were: (1) deciduous forest, (2) pines, (3) brush savanna, (4) grasslands, and (5) cropfields. Similarly, within home ranges habitat use was not proportional to availability (Wilk's $\lambda = 0.4786$, $P < 0.0001$). Within core areas (3rd-order selection), the order of preference was: (1) brush savanna, (2) deciduous forest, (3) pines, (4) grasslands, and (5) cropfields. Overall, habitat use relative availability at both the 2nd- and 3rd-order levels suggested that red squirrels preferred brush-savannas, deciduous forests, and pine plantations and avoided grasslands and cropfields (Figure 7). We found little evidence that red squirrels preferred pine plantations to deciduous forests; both habitat types were used heavily.

We performed compositional analysis using both SAS software and LeBan's Resource Selection software (Leban 1999). Results using the two methods differed only slightly in ranking habitat preferences. Consequently, we chose to use the Resource Selection Software for simplicity of interpretation.

At the outset of this study, we hypothesized that red squirrels would prefer forest patches with greater percent canopy cover. We tested this hypothesis by comparing canopy cover at sites where squirrels were radiolocated with randomly-selected sites. However, we found that canopy cover did not differ between occupied and random sites ($F = 2.41$, $df = 1$, $P = 0.121$).

DISCUSSION

Home range size, core overlap, and food caching

Summer home range size for all squirrels averaged 1.7 ha (SE = 0.24), and mean core area size was 0.4 ha (SE = 0.06). The size of both home ranges and core areas did not differ between males and females or adults and juveniles. Previous research has indicated that home range sizes of red squirrels vary across regions (Layne 1954; Smith 1968; Kemp and Keith 1970; Rusch and Reeder 1978; Gurnell 1984; Sullivan 1990; Vlasman and Fryxell 2002; Goheen and Swihart 2005). Our home range estimates are within the size ranges reported in previous studies (Table 6). Home ranges can differ due to season, location, habitat type, and calculation method, but there is value in making comparisons despite this. For instance, evaluating home range sizes in different regions and habitat types can provide insight into levels of habitat quality. A common assumption in wildlife biology is that home ranges tend to be smaller in high quality, resource-rich habitat, and larger in lower quality, suboptimal habitat. The main reason for this is that animals need a home range large enough to meet their life requisites (food, water, nest sites, breeding and brood raising ground, etc.), but no larger than necessary due to the costs associated with travel and defense.

Previous studies assessing red squirrel home ranges have categorized forests as either coniferous, mixed coniferous-deciduous, or deciduous. Obbard (1987) presented a review of territory and home range sizes of red squirrels across various habitat types. Based on this review, red squirrel home ranges tend to be larger in deciduous forests than in coniferous forests. Table 6 illustrates the size differential of red squirrel home ranges across various habitat types. These results suggest the lower habitat quality of deciduous forests when compared to that of coniferous forests. Based on these comparisons and assumptions, the red squirrels at ICSWA appear to occupy suboptimal habitat relative to that provided by the coniferous forests in the northern portion of the species' geographic range.

Across the red squirrel's range, it is difficult to characterize the species' territorial behavior and food-hoarding patterns with simple explanations (Hurly and Lourie 1997). Territoriality is thought to result from limited resources and cone crops in coniferous forests can be limiting for red squirrels. In the boreal forests of western North America, individuals establish exclusive territories and prepare for the winter by larder-hoarding cones in a central food cache (or midden) within their territories (Yahner 2003). Large quantities of cones can be stored there and individual red squirrels aggressively defend their midden from conspecifics and other animals (Smith 1968; Kemp and Keith 1970; Rusch and Reeder 1978; Gurnell 1984). Years in which the cone production is low cause food shortages, and the defense of middens may be critical to each animal's survival. Therefore, in these forests, year-round territorial behavior serves to protect a critical, seasonal resource (Smith 1968).

In contrast, the red squirrels in eastern boreal forests are usually territorial scatter-hoarders, burying and hiding cones and nuts in separate locations throughout their home range (Hurly and Robertson 1990; Dempsey and Keppie 1993). However, Hurly and Lourie (1997) also observed some larder-hoarding in this region.

In the deciduous forests of eastern North America, red squirrels scatter-hoard their food supplies and show no tendency to be territorial (Layne 1954; Dempsey and Keppie 1993). Unlike boreal forests, the deciduous and mixed forests of the eastern U.S. contain more diverse and dispersed food sources, likely reducing the benefits of territoriality.

Our observations of red squirrels in Illinois are consistent with these regional patterns of behavior. Scatter-hoarding was the typical method of caching food and core areas often overlapped, suggesting the absence of territoriality in the traditional sense of animals inhabiting exclusive areas and excluding conspecifics. We observed no evidence of larder-hoarding or territorial aggression aside from the occasional rattle-call. It was not unusual however to find piles of chewed walnuts beneath favorite foraging perches. Goheen (2002) observed an exception to this regional trend in Indiana where red squirrels occasionally constructed middens of black walnuts in deciduous woodlots.

Habitat selection and preferences

The home ranges of red squirrels on ICSWA were comprised predominantly of the forests, suggesting that within the study area, squirrels select deciduous forests and pine plantations as habitats. In turn, grasslands and agricultural fields were avoided. These patterns were expected, as red squirrels are forest-dwelling animals. However, at the finer scale (3rd order selection) of habitat preferences within the home range, the two analytical methods suggested no clear preference among forest types. X^2-tests suggested that red

squirrels preferred pine plantations and brush-savanna habitat types (both containing pine trees), but compositional analysis scored these habitat preferences somewhat differently with brush-savanna ranked as the most preferred type followed by deciduous forest, then pine plantation.

At the landscape scale, red squirrels used deciduous forest more than any other habitat type in Illinois. This result was somewhat surprising. Because of the species close association with conifers over most of their range, we hypothesized that the ICSWA population would show a preference for the pine plantations present on the site. However, our results indicate otherwise. Although pine plantations were available and used by individual squirrels, we found little evidence that they were preferred over the other forest types at either scale of selection. Our results are consistent then with those of Goheen and Swihart (Smith 1970), who reported that red squirrels showed no particular preference for pines, and readily used deciduous forests, in northern Indiana.

Although we did not quantify the relationship, an important habitat component in the deciduous woodlots occupied by red squirrels in Illinois appears to be mature black walnut (*Juglans niger*) trees. Many of the forested patches on ICSWA lack walnuts and red squirrels were rarely found in these. However, most occupied patches contained walnuts, as did the core areas of many individuals. We often found chewed walnut remains beneath favorite feeding perches used by these squirrels. Goheen and Swihart (2005) also found that walnuts were important to red squirrels in Indiana. We therefore speculate that the presence and abundance of red squirrels and mature walnut trees are positively correlated in this region.

The results of both X^2 testing and compositional analysis showed that brush-savannas are highly preferred by red squirrels at ICSWA even though this habitat type is relatively scarce. These savannas provide very thick ground cover and blackberries during the summer and squirrels used them as foraging areas and travel corridors. We were generally surprised by the large amount of time that red squirrels spent on the ground and the thick protective cover would provide shelter from predators. In addition, the extensive woody debris in this habitat type provided ground-level food caches and burrows used by red squirrels. Brush savannas contained sparse pine and deciduous trees, but it appeared that it was the structure of the understory and groundcover that most influenced the squirrels' preference for this habitat type.

Since red squirrels prefer the brush savanna, future management plans should include the provision of this habitat type. Our data and field observations suggest that its key components are likely thick underbrush, snags, downed wood, and mast-producing pines and/or walnuts. These components would provide the squirrels with protection from predation, nesting sites, additional food-hoarding sites, and sources of food. Silvicultural practices that include the thinning of canopy trees to stimulate the growth of woody understory, retention of walnuts and mast-producing species, and periodic prescribed burns and/or brushhogging to retard the development of midstory trees, would produce these features. During the summer season, the red squirrels appeared to spend considerable time in this habitat type.

Although pine plantations were not highly preferred over other forest types, we cannot dismiss the importance of conifers as a resource to red squirrels. The brush savanna preferred by squirrels contained sparse, mature pine trees as did the pine plantations

inhabited by some individuals. Two previous studies in Indiana indicated a positive relationship between red squirrels and conifers in that state and the authors speculated that conifers are important to the survival of these animals in the Midwest (Nupp 1997; Goheen and Swihart 2005). The two resource selection methods that we used showed conflicting results regarding the use of pine plantations. Therefore, even though the compositional analysis did not show a preference for pine plantations within home ranges, based on the X^2 results and our field observations, we believe that pines were an important component of core areas when present within the home range.

The home range of every radiocollared red squirrel contained some forest with dense understory and squirrels were rarely sighted on the ground where this was sparse. The most apparent function of dense understory is to provide the squirrels with protection from predation. Although we did not quantify the importance of a dense understory to Illinois' red squirrels, two seasons of radiotracking and behavioral observations lead us to believe that its presence contributes to the local persistence of this species. Another important habitat component appeared to be the presence of black walnuts. Red squirrels were often observed transporting and eating walnuts and it is evident that they provide an important food source. Of the animals we tracked, 7 had core areas comprised entirely of deciduous forest and each contained mature walnut trees. These nuts may be of particular importance because their heavy shells reduce their perishability compared with other food items (Goheen et al. 2003; Goheen and Swihart 2005).

Towards the end of our study, the IDNR had begun to girdle mature pine trees and remove plantations on the ICSWA because these trees are not native to the region. Concerns have been expressed that pine removal may reduce the distribution, abundance,

and persistence of red squirrels on ICSWA and other public lands. However, based on our results, it seems likely that red squirrels should be able to persist on the site, as long as deciduous woodlots are maintained with a walnut component; particularly if forest patches are interconnected with wooded corridors containing thick understories. Management to maintain or increase other habitat features likely to benefit these squirrels should also be considered. For example, maintaining mast-producing trees such as oaks and hickories (*Carya* spp.), cavity trees, snags, logs, and downed wood would likely improve habitat quality. Our study was not designed to assess habitat needs in the winter, but these features should provide winter food and protective shelter as well.

Presence and interactions with other Sciurids

Extensive and intensive live-trapping at ICSWA showed that low numbers of gray and fox squirrels were present and sympatric with red squirrels on the site. However, generally where red squirrels were abundant, very few gray and fox squirrels were present. we suspect that local habitat characteristics alter the competitive balance among these 3 species of tree squirrels. We did not witness direct interactions between any 2 species, so cannot speculate as to whether interference competition, exploitative competition, or both may influence the distribution and relative abundance of these species. In contrast, chipmunks were very abundant in local areas occupied by red squirrels. We were also impressed by how frequently red squirrels were found traveling or foraging on the ground. In this way, the behavior of red squirrels resembled that of chipmunks; and it would appear that the latter species could be important food competitors with red squirrels in this area during the summer. Both species are diurnal and primarily granivorous animals with

considerable overlap in their diet. This competition would be eliminated in the winter when chipmunks hibernate, while red squirrels stay active.

In conclusion, we found that red squirrels readily use deciduous forests in northeastern Illinois and that the brush savanna habitat type is used heavily within home ranges when available. Furthermore, our data suggest that mature walnut trees and a dense understory are important habitat components for these animals. Gray and fox squirrels were not abundant in forests used by red squirrels, but chipmunks and red squirrels appear to be competing for food in these habitats during the summer months. Our results further suggest that red squirrels are likely to persist at ICSWA in spite of the ongoing removal of pine trees. This is because they were not dependent on pines and were frequently found in forests lacking a pine component, provided that mast-producing walnuts and dense understory vegetation were present. Finally, we found little evidence of territorial behavior in this population; home ranges and core areas frequently overlapped and middens of pinecones or walnuts were not observed. These squirrels appear to be non-territorial scatter-hoarders.

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Table 6. Habitat composition of the ICSWA study site (habitat available) versus that within the home ranges (habitat used) during the summers of 2006 and 2007.

Habitat type	Study site composition (ha)	Proportion of study area	Observed composition of home ranges (ha)	Observed proportion of home ranges	Expected composition of home ranges (ha)	95% Bailey confidence intervals on observed home range proportions
Pine plantation	70	0.04	13.6	0.218	2.8	$0.201 \leq p_1 \leq 0.235$
Deciduous	775	0.44	41.3	0.592	30.3	$0.572 \leq p_2 \leq 0.612$
Grassland	421	0.24	4.7	0.042	16.5	$0.034 \leq p_3 \leq 0.051$
Cropfield	486	0.28	2.5	0.025	19.3	$0.019 \leq p_4 \leq 0.032$
Brush-savanna	3	< 0.01	6.9	0.123	< 0.1	$0.110 \leq p_5 \leq 0.137$
Total	1755		68.9		68.9	

Table 7. Habitat composition of the home ranges (habitat available) versus that within the core areas (habitat use) during the summers of 2006 and 2007.

Habitat type	Home range composition (ha)	Proportion of home ranges	Observed composition of core areas (ha)	Observed proportion of core areas	Expected composition of core areas (ha)	95% Bailey confidence intervals on observed core area proportions
Pine plantation	13.6	0.197	4.3	0.247	3.5	$0.230 \leq p_1 \leq 0.265$
Deciduous	41.3	0.599	10.2	0.554	10.8	$0.534 \leq p_2 \leq 0.574$
Grassland	4.7	0.068	0.4	0.013	1.2	$0.009 \leq p_3 \leq 0.019$
Cropfield	2.5	0.036	0.2	0.008	0.6	$0.005 \leq p_4 \leq 0.012$
Brush-savanna	6.9	0.100	2.9	0.178	1.8	$0.163 \leq p_5 \leq 0.194$
Total	68.9		18.0		18.0	

Table 8. Results of compositional analysis showing the relative preferences of red squirrels for each habitat type relative to the others at the 2nd-order level of habitat selection (study site composition versus home range composition). *T*-statistics and their corresponding *P*-values are given. Probabilities ≤0.05 suggest that the habitat type listed in the first column is significantly preferred over those listed in the other columns.

Rank	Deciduous	Pine plantation	Brush savanna	Grassland	Cropfield
#1 Deciduous	-	2.89 0.006	4.06 < 0.001	6.76 < 0.001	9.55 < 0.001
#2 Pine plantation		-	0.97 0.337	1.96 0.057	4.16 < 0.001
#3 Brush-savanna			-	1.89 0.067	2.80 0.008
#4 Grassland				-	1.85 0.071

Table 9. Results of compositional analysis showing the relative preferences of red squirrels for each habitat type relative to the others at the 3rd-order level of habitat selection (home range composition versus core area composition). *T*-statistics and their corresponding *P*-values are given. Probabilities ≤0.05 suggest that the habitat type listed in the first column is significantly preferred over those listed in the other columns.

Rank	Brush savanna	Deciduous	Pine plantation	Grassland	Cropfield
#1 Brush savanna	-	1.77 0.083	2.04 0.048	4.29 < 0.001	4.35 < 0.001
#2 Deciduous		-	0.51 0.610	2.60 0.013	2.15 0.038
#3 Pine plantation			-	1.76 0.086	1.86 0.070
#4 Grassland				-	0.095 0.925

Table 10. Territory/home range sizes of red squirrels in various forest types.

Forest Type	Location	Average territory/home range size (ha)	Season	Method	Source
Coniferous	British Columbia, CA	0.79	Apr.-Sept.	Observations	Smith 1968
Coniferous	Alberta, CA	0.24-0.66	Year-round	Observations	Rusch & Reeder 1978
Coniferous	Colorado	0.56	Sept.-Nov.	Observations	Gurnell 1984
Coniferous	British Columbia, CA	0.63	Year-round	MCP	Sullivan 1990
Coniferous	Ontario, CA	0.79	May-July	MCP	Vlasman & Fryxell 2002
Mixed	Alberta, CA	0.4-0.8	June-Aug.	Observations	Kemp & Keith 1970
Deciduous dominant	New York	1.1-2.44	June-Aug.	Observations	Layne 1954
Deciduous dominant	Indiana	0.88-1.03	Year-round	90% kernel	Goheen & Swihart 2005
Deciduous dominant	Illinois	1.7	May-Aug.	90% kernel	Hanson 2007

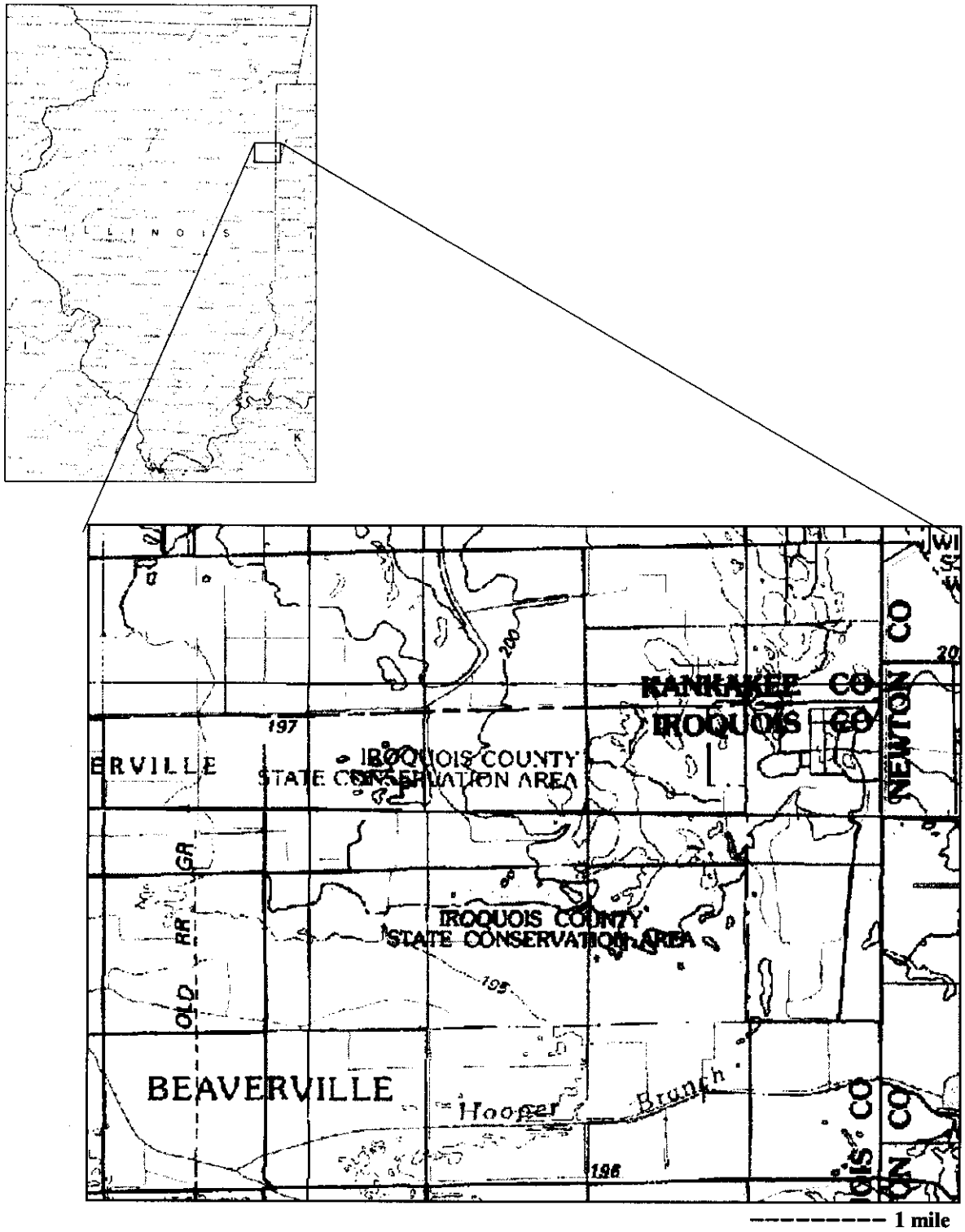
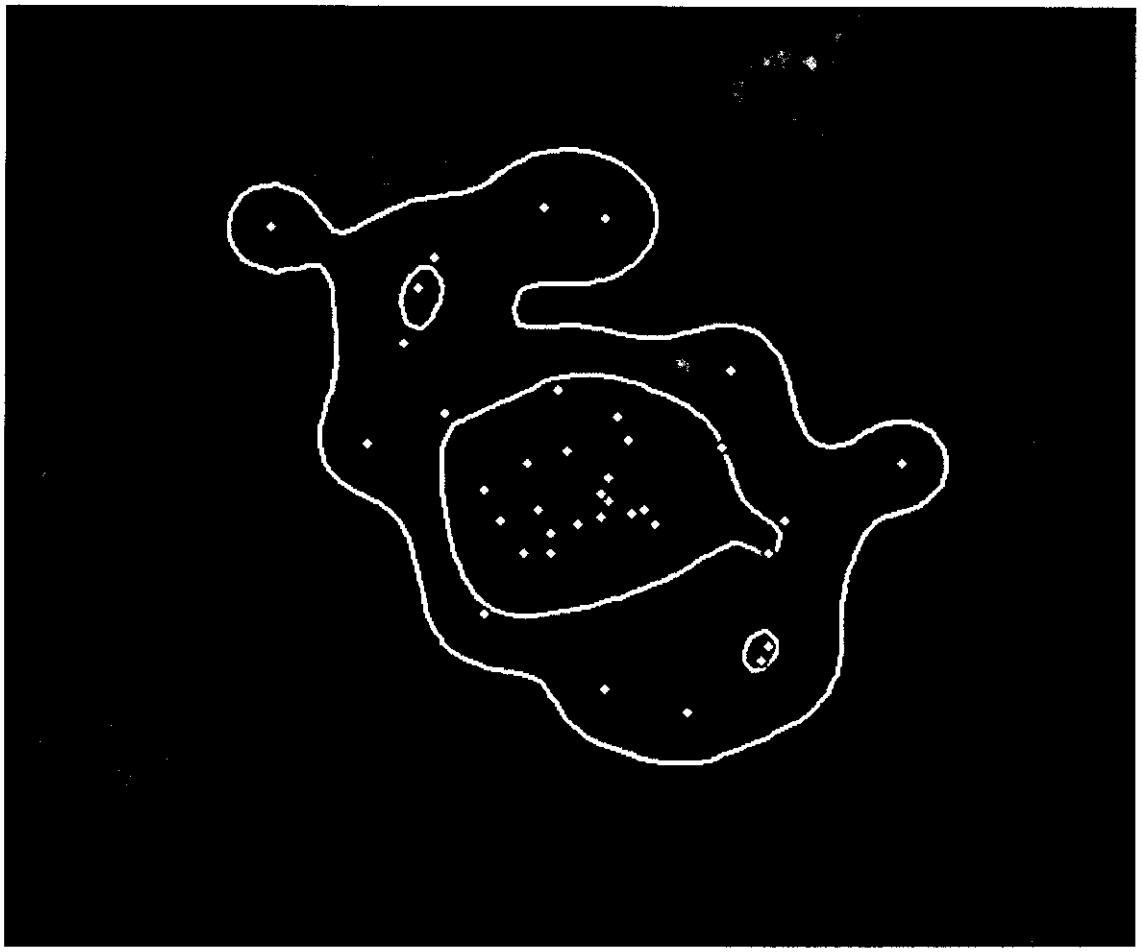


Figure 7. Map of the Iroquois County State Wildlife Area in northeastern Illinois located ~ 2 km from the Indiana border (Illinois Geospatial Clearinghouse, 2007).



---------- **100 meters**

Figure 8. An example of a fixed kernel home range and core area estimation. The dots represent the radiolocations of an individual red squirrel. The 90% home range is the region encompassed by the outer polygon. The 50% core area is the area outlined by the 3 inner polygons.

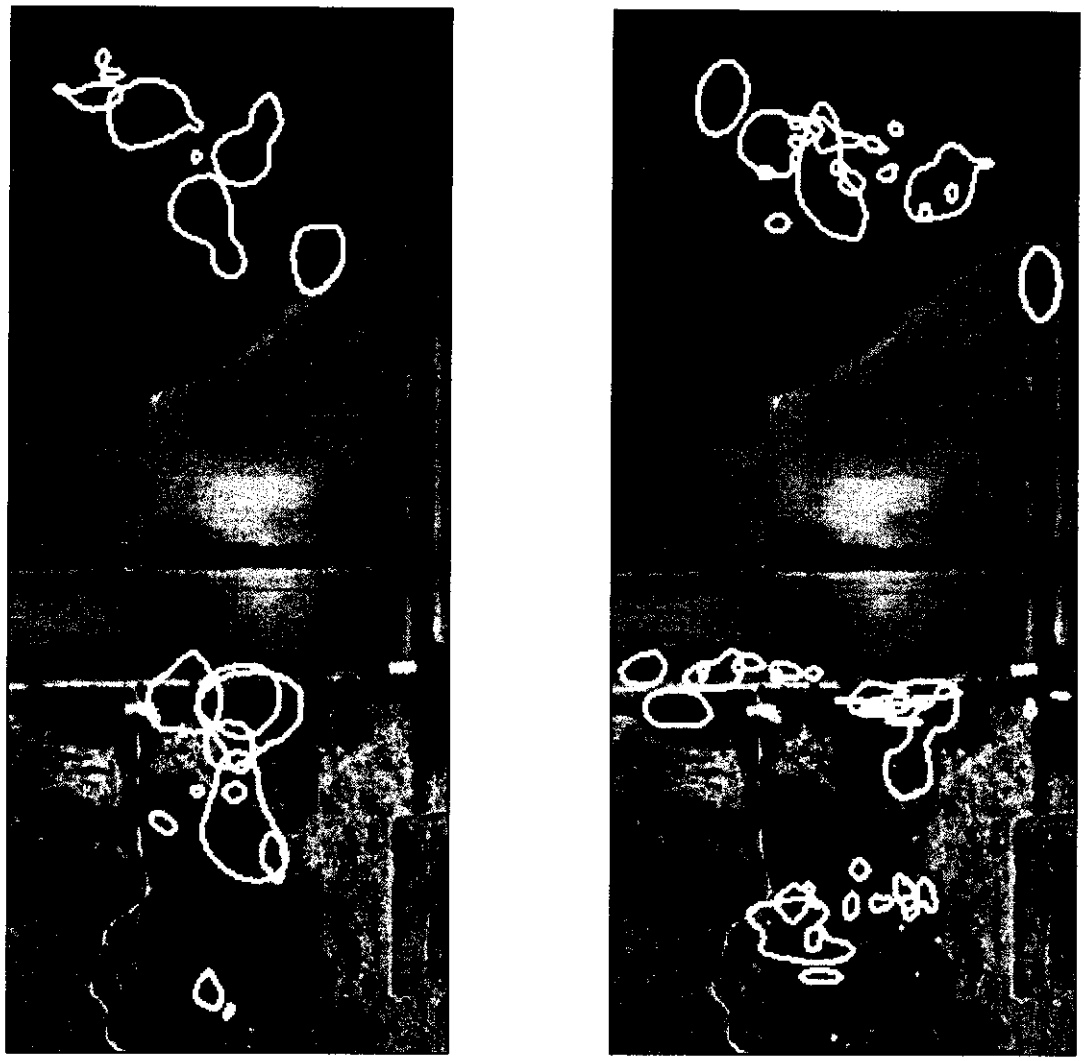


Figure 9. Images showing overlap among core areas of individual red squirrels. The left image is from the 2006 field season; the image on the right is from 2007.

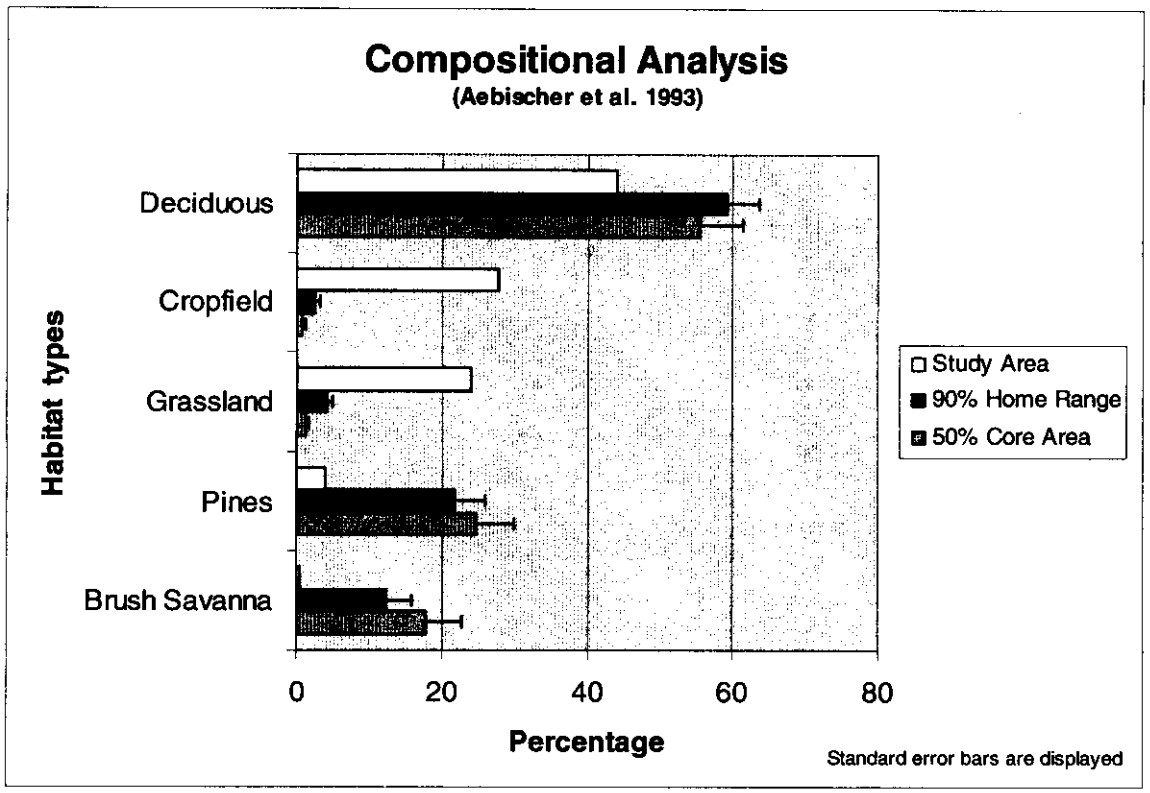


Figure 10. Habitat composition analysis of the study area, home ranges, and core areas used by red squirrels at ICSWA. Second-order habitat selection can be ascertained by comparing the composition of the study area (habitat available) to the composition of the home ranges (habitat used). Third-order selection can be observed by comparing the composition of the home range (habitat available) to that of the core area (habitat used).

JOB 3. EVALUATE LANDSCAPE-LEVEL EFFECTS ON THE CURRENT AND FUTURE DISTRIBUTION OF RED SQUIRRELS IN ILLINOIS

DISCUSSION

Red squirrels in Illinois inhabited the northern one-third of the state before European settlement and now exhibit a restricted range. Our playback surveys and interviews suggest that the geographic range of the species has expanded by ~50 km to the west during the past 30 years. Furthermore, current patterns of patch occupancy indicate that the species is neither particularly area-sensitive nor incapable of dispersing through fragmented Midwestern habitats. Once thought to be a coniferous forest specialist, we now know that red squirrels inhabits both large and small, contiguous and isolated deciduous forests, pine plantations, and mixed hardwood-pine forests in northeastern Illinois and Indiana. Furthermore, our field surveys and genetic analyses indicate clearly that the Illinois population is not a disjunct or remnant population, but rather is primarily the result of a range expansion from Indiana and southern Michigan. This range expansion, combined with an apparent one-time transfer of Minnesota squirrels into the area, has produced an admixed genetic population in Illinois that is differentiated from the larger Midwestern population only by its mixed Indiana and Minnesota ancestry.

Based on the best ecological and genetic information available, we hypothesize that red squirrel populations is slowly expanding in the state. Agricultural fields, grasslands, roadways, and rivers do not appear to be barriers to movement. We found squirrels in woodlots surrounded by corn and soybean fields, on both sides of Interstates 57 and 55, and on both sides of the Kankakee and Iroquois Rivers. Range expansion appears to be occurring primarily (but not exclusively) along the riparian forests of the Kankakee and

Iroquois Rivers and these serve as critical habitat and corridors for movement. Seven of 11 woodlots recently occupied by red squirrels were within 2 km of these rivers and their tributaries.

Red squirrels inhabit deciduous forests, both with and without a coniferous component, in Illinois. The understory in inhabited woodlots varied from sparse to very dense. However, two factors were common to all occupied woodlots: (1) the presence of mature walnut trees, and (2) the absence or scarcity of gray squirrels. Occupied patches varied from 5.4 ha to >1,500 ha in size and from narrow to wide in shape. We found no evidence that patch size or shape influenced occupancy. Woodlots <20 ha in size were occupied suggesting that the species is not particularly area-sensitive, as were patches >2 km from the nearest apparent habitat indicating that they are capable of dispersing across stretches of non-forested landscape. For example, we found red squirrels occupying a small, isolated woodlot at Goose Lake Prairie surrounded by more than 1,000 ha of prairie and >1.5 km from the nearest forested corridor.

Summer home ranges averaged 1.7 ha (SE = 0.24) in size and core areas averaged 0.4 ha (SE = 0.06). The size of home ranges and core areas did not differ between males and females or adults and juveniles. Home ranges were larger than previously reported for red squirrels occupying prime habitats. We interpret this to mean that northeastern Illinois provides only marginal habitat quality for this species. We found little evidence of territorial behavior in this population. Home ranges and core areas frequently overlapped and middens of pinecones or walnuts were not observed. These squirrels appear to be non-territorial scatter-hoarders.

Relative to the composition of the study area as a whole, home ranges were comprised predominantly of deciduous forest and pine plantations. Grasslands and agricultural fields were avoided. At the finer scale of habitat use within home ranges, we found no clear preferences among forest types. X^2-tests suggested that squirrels preferred brush-savanna and pine plantations to deciduous forests, but compositional analysis ranked brush-savanna as the most preferred, followed by deciduous forest, then pine plantation. Individuals of both sexes spent considerable time on the ground foraging in thickets and dense understory. Field observations showed that mature walnut trees and a dense understory are important habitat components for these animals. Gray and fox squirrels were not abundant in forests used by red squirrels, but chipmunks are likely competitors for summer food and burrows.

In summary, management practices that serve to maintain riparian corridors, retain mature mast-producing hardwoods (particularly walnuts), and provide dense understory thickets will help to conserve red squirrels. While non-native pine plantations are used by squirrels in northeastern Illinois, the presence of red squirrels in deciduous forests lacking pines suggests that the removal of these plantations on public lands does not threaten the persistence of red squirrels in the region.