Genetic relatedness in winter populations of seasonally gregarious southern flying squirrels, *Glaucomys volans*

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Abstract:

Southern flying squirrels (*Glaucomys volans*) face a dilemma: winter aggregation is beneficial for thermoregulation but costly due to nest mates pilfering stored food in the home area and the tendency for groups to attract predators. Living with kin in winter aggregations may mitigate these deleterious effects because if an individual dies, its stored food can be beneficial to relatives, thereby increasing inclusive fitness. Southern flying squirrels from 7 populations and a captive colony were genotyped at 6 microsatellite loci. We calculated group mean relatedness and dyad relatedness within groups. In the wild, winter populations were found to be more highly related than expected by chance. Fifty-seven percent of animals were associated with a highly related individual in their winter aggregation. We show that southern flying squirrels have a preference for relatives as winter nest mates.

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Group living and sociality occur when these behaviors provide fitness advantages to the social animals (Alexander 1974; Lacey 2004). Benefits of group living can include access to additional food or shelter resources, warnings about the presence of predators, access to potential mates, and cooperation in rearing young (Alexander 1974; Dickinson and Koenig 2003). Potential costs of group living to individual fitness are a reduction of individual reproductive opportunities and generalized reduction of resource availability. Additionally, the presence of many individuals in the immediate area can attract predators and concentrate parasite loads (Alexander

1974; Dickinson and Koenig 2003; Lacey 2004; Sinervo and Clobert 2003). Genetic relatedness is thought to mitigate these adverse aspects of group living in many organisms by increasing inclusive fitness. Forms of nepotism, kin recognition, kin discrimination, and genetic discrimination have been found in organisms as diverse as the cellular slime mold (*Dictyostelium purpureum*—Mehdiabadi et al. 2006), the side-blotched lizard (*Uta stansburiana*—Sinervo and Clobert 2003), and African elephant (*Loxodonta Africana*—Archie et al. 2006). Most of the well-documented social systems are those that occur in species that are gregarious year-round or whose seasonal fluctuations in aggregation behavior are mediated by breeding or hibernation (Blumstein and Armitage 1999; Holekamp et al. 1997; Sherman 1981).

Southern flying squirrels (*Glaucomys volans*) present an interesting model for exploring seasonal changes in sociality because their patterns of seasonal aggregations are not closely associated with breeding or hibernation. They are small, nocturnal, arboreal, nonhibernating gliding squirrels that inhabit temperate hardwood and mixed forests in eastern North America (Dolan and Carter 1977; Layne and Raymond 1994). Southern flying squirrels have a fairly widespread but patchy distribution throughout their range, preferring mature forest with many nest sites, predominantly tree cavities, and plentiful storable food, such as hickory nuts (*Carya*spp.) and acorns (*Quercus* spp.—Bendel and Gates 1987; Doby 1984; Holloway and Malcolm 2007). Like other North American tree squirrels, southern flying squirrels do not migrate and are active throughout the winter; therefore they must contend with harsh winter conditions in most of their range.

Winter aggregation and well-constructed nests reduce thermoregulatory costs in southern flying squirrels (Merritt et al. 2001; Muul 1968; Stapp 1992; Tompkins 2003; Weigl 1978). Declining photoperiod and lower temperatures initiate food-storing activities and the formation of winter aggregations. Groups average from 5 to 20 individuals, and aggregation size fluctuates during the course of the winter, tending to be larger in colder weather (Muul 1968). Seasonal nest defense by pregnant and postpartum females initiates disaggregation of groups in the spring (Dolan and Carter 1977; Muul 1969, 1970; but see Madden 1974). Like some other tree squirrels and the social marmots, home ranges of individual southern flying squirrels significantly overlap (Blumstein and Armitage 1999; Carraway and Verts 1994; Dolan and Carter 1977; Koprowski 1994, 1996; Nash and Seaman 1977).

Due to the home-range overlap found in southern flying squirrels (Bendel and Gates 1987) we suggest a high probability that individual animals will encounter and recognize each other, which may facilitate formation of winter aggregations. As individually scatter-hoarding granivores that live in groups with overlapping home ranges, the squirrels concentrate vast quantities of tree mast in the home range (Weigl 1978). The squirrels do not defend their caches. Therefore, although the individual squirrel that stores a particular food item has a marked advantage over other animals in finding that food item, it must contend with other animals, including nest mates opportunistically pilfering its cached food (Winterrowd 2001; Winterrowd and Weigl 2006). This opportunistic pilfering is greatly facilitated by olfaction (Winterrowd and Weigl 2006) and in no way implies intentional cache sharing.

Southern flying squirrels face a dilemma. In winter, group living is advantageous for energy conservation but can be deleterious to individual survival due to food losses, predator attraction,

and transmission of internal parasites (Weigl 1978; Wetzel and Weigl 1994; Winterrowd and Weigl 2006). Living preferentially with kin may mitigate these effects because individual squirrels can gain access to the local food resources their nest mates have stored within the home range. Even if an individual dies, the food it stored in its home area can benefit its nest mates. Additionally, if relatedness between interacting individuals is higher than background relatedness levels in the population, altruistic behaviors and the resulting benefits of sociality may be perpetuated genetically; that is, benefits derived from inclusive fitness (Alexander 1974; Griffin and West 2002; Kerth et al. 2002; West-Eberhard 1975).

During 5 years of checking nest boxes in Florida Layne and Raymond (1994) found that at least 16% of southern flying squirrel aggregations contained a minimum of 1 pair of known 1st-order related adults. Winterrowd et al. (2005) found that in late spring and summer (March through June) relatedness (r) of mixed-age squirrel nest groups was high (family groups consisting of females and nursing pups, with r = 0.5; subadult groups consisting of juveniles and adults, with r = 0.38), whereas adult-only aggregation relatedness was low (r = 0.03). These late spring and summer nest groups were from an area of the South Carolina sandhills managed for red-cockaded woodpeckers (*Picoides borealis*) by habitat modification and flying squirrel removal. Despite the high degree of disturbance due to management, one-third to one-half of all spring mixed-age nest groups were related in this springtime, highly managed situation (Winterrowd et al. 2005). The relatedness composition of winter aggregations is unknown, although examination of behavioral data from captivity suggests a preference for relatives in winter (Thorington 2008).

If southern flying squirrel winter aggregations are mediated by kinship, wild squirrels from the same geographic population or nest group should display a higher relatedness to each other than to squirrels from other populations and nest groups. Given the southern flying squirrel's dilemma and the observation of low relatedness in summer adult-only groups, our goal was to determine whether wild southern flying squirrels are nesting preferentially with kin in winter. Specifically we addressed whether squirrels from the same location display high mean group relatedness, and whether these squirrels lived with related individuals during winter aggregation. To this end we tested genetic relationships—both group mean relatedness and pairwise (dyad) relatedness—in wild-caught, geographically distinct populations using micro-satellite markers. Microsatellites are short 1- to 5-base-pair (bp) motifs that form 1- to 40-bp repetitive sequences. They are noncoding, highly variable, and mutate quickly, making them good markers to measure relatedness within and between generations of animals (Karp 2005).

Materials and Methods

Samples of DNA were collected from wild-caught southern flying squirrels in 7 geographic regions (Table 1). These include locations in Alabama (AL), Georgia (GA), South Carolina (SC), North Carolina (NC Piedmont B, NC Piedmont H, and NC Mountain), and Pennsylvania (PA). Squirrels living in the outdoor captive colony at Wake Forest University also were sampled. Tissue was collected either as small ear clips or dried hair follicles. Samples from the AL, GA, SC, NC Piedmont H and B, and NC Mountain groups, and the captive colony consisted of ear clips taken by cutting a small notch (3–5 mm) in the rim of the ear. Sample tissue was immediately immersed and stored in 95% ethanol, or in the case of the NC Mountain population, in lysis buffer. Samples from PA were groups of approximately 30 hairs with attached follicles

pulled from the tip of the tail and stored taped to an index card at ambient temperature. All tissue collection and squirrel care conformed to guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998; Gannon et al. 2007) and regulations of the Wake Forest University Animal Care and Use Committee (protocols A03-117 and A06-212).

Table 1. Locations, habitat type, and habitat quality where each southern flying squirrel population sample was taken. Habitat quality is a qualitative estimate based on the sample collector's observations and descriptions of available nest and mast trees. Number of animals = total population sample (subsamples). State abbreviations used are: AL = Alabama, GA = Georgia, NC = North Carolina, PA = Pennsylvania, and SC = South Carolina.

Location	No. animals[a]	Habitat Description	Quality	Group collection type
PA, Monroe County	8	Hardwoods with conifer pockets	High	1 nest-box group
NC Piedmont (H), Forsyth County	9	Suburban attics, older hardwoods	High	Trapped over the course of 6 weeks
NC Piedmont (B), Forsyth County	12		High	Livetrapped
GA, Baker County	11 (4,7)	Pine plantation	Low	Multiple red-cockaded woodpecker boxes; shared nesting unknown; animals are from 2 years of trapping
NC Mountains, Haywood County	15 (2, 3, 4, 6)	Altitudinal edge of range, red oak conifer forest	Low	4 nest-box groups
AL, Washington County	7	Rural, mixed woods and agriculture	Medium	Livetrapped
SC (HP), York County	2	Young successional forest with some older field-grown mast trees	Medium	2 nest boxes, each contained a lactating female and her 2 (~1- to 2-day-old) pups; only the adult females were sampled

[a] One additional animal was caught in the North Carolina Piedmont, but as a singleton her DNA was used only for background allelic frequency.

All wild-caught animals not resident in the captive colony were from known nest boxes or tree cavities or were livetrapped in suburban attics as problem animals (Table 1). The Wake Forest University captive colony consisted of 61 squirrels at the time of tissue sampling. Twelve of these animals (NC Piedmont B) were trapped in a Winston-Salem residence. Twenty adult animals in the colony were of mixed origin. Prior to 2002 records of origin specific to each animal were not kept for most animals. Wild-caught squirrels were either from the Savannah River Ecology Lab at the Savannah River Site, Georgia, Archbold Biological Station, Florida, or locally caught around Winston-Salem, North Carolina. From 2002 thorough spring 2007 an active breeding program was conducted, and 29 known-relationship offspring survived to adulthood representing 4 generations of squirrels. These offspring also were genotyped (Thorington 2008).

The term group is used generically and may refer to various sets of animals in different conditions. The term geographic population is used for animals collected within an approximately 800-m radius of a particular point and may include animals from a single aggregation or attic, collected from multiple aggregations in a nest-box line, or collected over

multiple years. The term subpopulation refers to a sample from a single aggregation in a geographic population or the subset of squirrels sampled in a single year. The term nest group refers to squirrels sampled while sharing a dray or nest cavity. Two of the geographic populations included subpopulation samples. The Georgia samples were collected over a span of 2 years. The NC Mountain population consisted of 4 nest groups collected along 2 box-line transects (Table 1).

Collection habitats were categorized qualitatively as high, medium, or low quality based on the description of each site (Bendel and Gates 1987; Doby 1984; Weigl 1978; Table 1). Stable habitats with plentiful and annually reliable storable tree mast were considered high quality. Low-quality habitat had few mast-producing trees and few potential nest cavities. Medium-quality habitats were intermediate in these respects; that is, mast trees were present in numbers but trees were young, or nest trees were scarce although food was readily available. Pine plantation monocultures managed for wood production or red-cockaded woodpeckers are considered low-quality habitat and generally would not have a good mast crop, and high altitude–northern latitude mixed forest might have similar food shortage problems. Although high-quality habitat, such as hardwood or mixed forests and forested suburbs, varies annually, it generally has more consistent mast availability. Even in bad years suburbs often contain supplemental anthropogenic foods such as birdseed mixes (Prange et al. 2004; Sauter et al. 2006).

Microsatellite analysis.—Samples from 114 squirrels—65 wild-caught in 7 populations (Table 1) and 49 captive animals—were analyzed. Total genomic DNA was extracted using a DNeasy Tissue Extraction Kit (QIAGEN, Valencia, California). Six microsatellite loci were amplified by polymerase chain reaction using published protocols (Table 2). For a sample of the reactions amplification of the correct fragment was verified by removing 5 µl of the polymerase chain reaction product to visualize in a gel. Polymerase chain reaction products were loaded into a MegaBACE 500 sequencer with an in-lane standard (ET400-R; GE Healthcare, Piscataway, New Jersey), and allele sizes were determined using Fragment Profiler (GE Healthcare). These 6 loci were used because they amplified well and were polymorphic for our samples. Only 9 loci have been isolated specifically for the southern flying squirrel (Fokidis et al. 2003; Winterrowd et al. 2005). Of these we successfully amplified 5 (Table 1). Additionally, 7 loci have been isolated for the northern flying squirrel (Zittlau et al. 2000) and 1, GS2, worked reliably for us. We found no differences in amplification of hair versus tissue samples. This was tested with tissue collected in both ways from captive squirrels.

We calculated genetic diversity indices in Cervus 2.0 (Marshall et al. 1998). When tested using GENEPOP (Raymond and Rousset 1995) the 6 loci did not deviate from Hardy-Weinberg equilibrium. We calculated pairwise relatedness estimates in KINSHIP 1.2 (Goodnight and Queller 1999). Genetic pairwise relatedness estimates (r_M) are continuously distributed from −1 to +1. Negative values result when dyads share fewer alleles than expected at random, which roughly corresponds with a biological relatedness of 0 (Metheny et al. 2008b). We used all 65 wild-caught animals and 20 adults of unspecified origin from the captive colony to determine background allele frequency. The other 29 animals from the captive colony were offspring of either the original colony animals or the NC Piedmont B group or crosses between squirrels in these groups. The NC Piedmont B group resided in the captive colony after being trapped out of

an attic in 2001. The 29 offspring were excluded from the allele frequency calculation to avoid overestimating or underestimating the frequency of rare alleles.

Analyses.—For each geographic population and each subpopulation we used a randomization test to determine whether the observed group of squirrels was more closely related than expected by chance (Metheny et al. 2008b). For each observed group we resampled the observed number of individuals in the group from the 85 adult squirrels 999 times to generate a random distribution of average relatedness values. For example, if 7 individuals comprised the focal group, we randomly selected 999 groups consisting of 7 individuals each and calculated a mean relatedness value for each group. We then compared the observed average relatedness to this randomly generated distribution. Observed values that fell in the highest 10% of the random distribution were considered statistically significant. We used $P \leq 0.10$ as the criterion because it was biologically salient, allowing us to detect high mean levels of kinship or a kin-based core in a given aggregation. We report exact *P*-values to facilitate interpretation. At the 10% ($P = 0.10$) level a group where 35–45% of squirrels are highly related is considered significant; for example, the presence of 3 siblings in the group of 7 described above would be significant. High relatedness is defined as Hamilton's r (r_H) ≥ 0.25 (Hamilton 1964). At a 5% ($P = 0.05$) level the same population where 35–45% of animals are related has the potential to be nonsignificant. Mean group or colony relatedness is not always the best predictor of highly related dyads or other subgroup structure within groups of animals (Kerth et al. 2002). Therefore, we also examined dyadic r_M-values within each group to detect highly related dyads within aggregations.

Dyad relatedness.—The captive colony included known-relationship squirrels, and detailed pedigree information existed for most animals. In 2002 when the active breeding program began, the captive-colony adults were not related. We used known relationships of 176 related dyads and 100 unrelated dyads to inform our interpretation of r_M for wild-caught pairs (our pairwise relatedness estimates based on microsatellite genotyping). Pedigree relatedness (r_H) ranges from 0 to +1 with 0 being unrelated and 1 being genetically identical (Hamilton 1964). This scale assigns the theoretical r_H-value of 0.5 to full-sibling dyads and parent–offspring dyads (1-step dyads; $n = 65$, $r_M\overline{X} = 0.457 \pm 0.150$). Half-sibling, grandparent-grandchild, and other 2-step dyads are defined as $r_H = 0.25$ ($n = 83$, $r_M\overline{X} = 0.289 \pm 0.210$). Three-step dyads are assigned an $r_H = 0.125$ ($n = 26$, $r_M\overline{X} = 0.130 \pm 0.140$). Unrelated pairs are assigned an $r_H = 0$ ($n = 100$, $r_M\overline{X} = 0.006 \pm 0.201$). The relatedness categories used to compare the percentage of related animals and dyads within each wild-caught population sample were generated from these colony-generated observed ranges. The highly related dyads ($r_H = 0.5$ range) were easily separated from the $r_H = 0.125$ and $r_H = 0$ dyads (Fig. 1). Therefore, we placed dyads into 3 increasingly inclusive categories to generate group relatedness estimates measured in percentage of dyads based on the relatedness of those dyads. The conservative (highly related) estimate is roughly equivalent to the randomization test. From these comparisons we were able to categorize our r_M for wild-caught groups into categories of pairwise relatedness with 1 or more animals in their wild-caught group. High $r_M \geq 0.270$ includes all $r_H = 0.5$ range animals and the $r_H = 0.25$ range animals that do not fall into the $r_H = 0.125$ range. Medium $r_M \geq 0.130$ includes all of the dyads with an r-value above the mean for $r_H = 0.125$ animals. Low $r_M \geq 0.079$ includes all dyads with an r_M-value at or above 1 *SD* below the $r_H = 0.25$ mean. To estimate the percentage of dyads related above those levels within each group we calculated 3 estimates: the conservative estimate is simply the percentage of high r_M dyads; the midrange estimate is all dyads at and above $r_M \geq 0.130$ and

therefore includes the conservative estimate; and the liberal estimate includes all dyads at $r_M \geq$ 0.079, and again the previous categories are included.
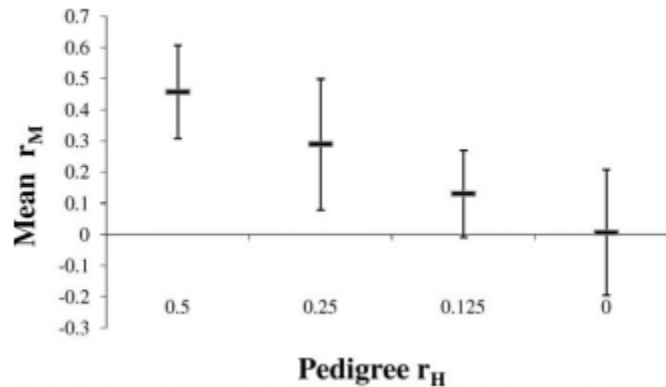

Fig. 1. Mean microsatellite DNA relatedness (r_M) of pedigree r_H-value category dyads (see text for explanation of Hamilton's relatedness, r_H). The horizontal dash is the observed r_M-value for each category. Error bars represent 1 *SD*. These means and *SD*s were used to calibrate dyad relatedness within the wild-caught populations.

Results

We generated microsatellite genotypes for 114 animals from 7 wild-caught populations and the captive colony. The wild-caught populations ranged in size from 2 to 12 squirrels. The 6 loci were polymorphic, with the number of alleles per locus ranging from 8 to 17 with a mean of 11.2 (Table 2). The number of alleles per locus was similar between the geographic populations and the captive colony. Two alleles, 1 from SFS-03 (217) and 1 from SFS-15 (099), were present only in 3 populations, but those 3 populations spanned the geographic range (Thorington 2008).

Table 2. Microsatellite variation in *Glaucomys volans* for all 6 loci used. T_a = annealing temperature, H_o = observed heterozygosity, H_E = expected heterozygosity.

Locus	MgCl$_2$(mM)	T_a(°C)[a]	No. alleles	Allele range (base pairs)	Null allele frequency	H_o	H_E	Source species	Citation
SFS-03	2.5	68/50	9	215–241	0.0345	0.647	0.607	*G. volans*	Fokidis et al. 2003
SFS-04	4.0	68/50	17	123–157	0.0201	0.889	0.847	*G. volans*	Fokidis et al. 2003
SFS-07	3.0	63/45	8	229–257	0.0126	0.776	0.753	*G. volans*	Fokidis et al. 2003
SFS-14	3.0	63/45	10	141–163	0.042	0.769	0.706	*G. volans*	Fokidis et al. 2003
SFS-15	3.0	63/45	10	99–117	0.0312	0.831	0.776	*G. volans*	Fokidis et al. 2003
GS-10	2.5	68/50	13	188–222	0.034	0.874	0.812	*G. sabrinus*	Zittlau et al. 2000

[a] Polymerase chain reaction amplification was done using a touchdown protocol that included a 4-min denaturation cycle at 95°C; 9 cycles of 45 s at 95°C, 2–4 min at either 68°C or 63°C with a decrease of 2°C per cycle, and 1 min at 70°C; 30 cycles of 45 s at 95°C, 2 min at 50°C or 45°C, and 1 min at 70°C; and a final extension of 5 min at 70°C.

Average relatedness of geographic populations.—Using the randomization test, 3 of the 7 wild-caught populations had higher average relatedness than predicted by chance ($P \leq 0.10$, based on 1,000 randomizations). These populations were NC Piedmont H ($r_M\bar{X} = 0.042$, $P = 0.080$), NC Piedmont B (mean $r_M = 0.041$, $P = 0.055$), and PA (mean $r_M = 0.048$, $P = 0.088$; Fig. 2; Table 3). The NC Mountain (mean $r_M = -0.021$, $P = 0.624$) and GA (mean $r_M = 0.022$, $P = 0.127$) populations as a whole were not more related than expected at random; however, they contained

subpopulations that had higher average relatedness than predicted by chance (NC Mountain subpopulation $n = 4$, mean $r_M = 0.118$, $P = 0.068$; GA 2007 $n = 7$, mean $r_M = 0.090$, $P = 0.023$; Table 3). The sample of the population from Hilton Pond, South Carolina, consisted of 2 females that had bred in the very early spring and therefore were no longer with their winter nest mates. On 7 March each was lactating and had a litter of 2 pups that were 1–3 days old (Hilton 2007). Excluding the Hilton Pond population from the relatedness analysis due to small sample size and group composition leaves 6 populations. Half (3) of these populations, and 2 additional subpopulations, were more related than predicted by chance alone, suggesting that more than half of winter nest groups were more related than would be expected with random assortment of individuals into winter aggregations. The 3 geographic populations that are significantly related are from high-quality habitat (Table 1). In contrast, the populations that are from low-quality habitat showed more variation in relatedness based on the randomization test.
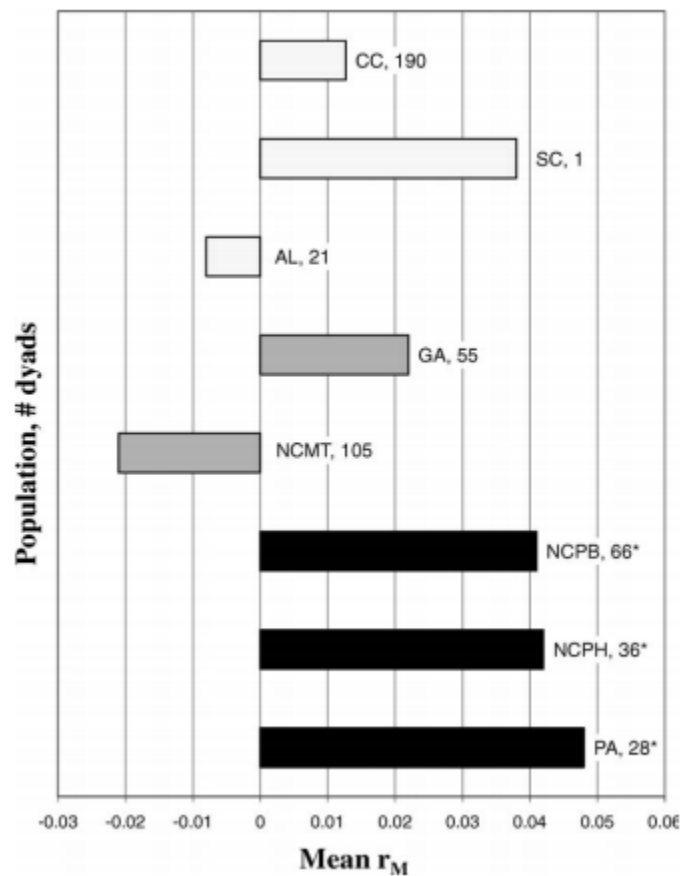

Fig. 2. Mean relatedness (r_M) of 7 wild-caught populations and the captive colony. An asterisk (*) indicates $P \leq 0.10$. Codes at the ends of the bars indicate the population in question followed by the number of dyads in that population. PA = Pennsylvania group, NCPB = North Carolina Piedmont B group, NCPH = North Carolina Piedmont H group, NCMT = North Carolina Mountain group, SC = South Carolina group, GA = Georgia group, AL = Alabama group, and CC = captive-colony adults. Black bars are those populations that as a whole are significantly related; gray bars are populations that contain significantly related subpopulations; and white bars are populations that are not significantly related. Habitat quality estimates are high = PA, NCPB, NCPH; medium = AL and SC; and low = GA and NCMT.

Table 3. Relatedness of wild-caught populations of southern flying squirrels shown in average relatedness (r_M) for each group, and percentage of dyads estimates for each group (conservative $r_M \geq 0.270$, midrange $r_M \geq 0.130$, liberal $r_M \geq 0.079$). Population abbreviations are: PA = Pennsylvania, NC = North Carolina, GA = Georgia, AL = Alabama, and SC = South Carolina.

Population	$r_M X$	Group size in		Relatedness (%) of dyads		
		No. animals	No. dyads	Conservative	Midrange	Liberal
PA	0.048*	8	28	14.3	32.1	39.3
NC Piedmont (H)	0.042*	9	36	19.4	27.7	44.4
NC Piedmont (B)	0.041*	12	66	16.6	24.2	31.8
GA All	0.022	11	55	10.9	29.1	40
GA 2006	−0.031	4	6	0	16.6	33.3
GA 2007	0.090*	7	21	19	38.1	47.6
NC Mountain All	−0.021	15	105	11.4	21.0	30.5
NC Mountain	−0.009	2	1	0	0	0
Subgroups (nest-box based)	−0.082	3	3	0	0	33.3
	0.118*	4	6	16.6	50	83.3
	−0.047	6	15	6.7	13.3	26.7
AL	−0.008	7	21	19	33.3	33.3
SC (HP)	0.038	2	1	0	0	0

*$P < 0.10$.

Dyad relatedness within wild-caught populations.—A minimum of 57% of animals were highly related ($r_M \geq 0.270$) to an individual within their own population. The conservative estimate showed that the percentage of highly related dyads ranged from 10.9% (GA All) to 19.4% (NC Piedmont H; Table 3). At the most liberal threshold $r_M \geq 0.079$, 87.5–100% of animals were highly related to an individual in the geographic population, and 30.5–44.4% of dyads in each population were highly related (Table 3). Six of the 7 populations contained a minimum (conservative estimate) of 4 ($\geq 11\%$) highly related dyads.

Discussion

Southern flying squirrels nest with kin in winter, and nest groups contain a kin-based core group supplemented with nonrelatives. Half of the observed geographic populations had a higher than expected mean relatedness. More than half of our populations showed a considerable degree of relatedness between dyads in their nesting aggregations, with all groups containing 11–45% related dyads. Additionally, although the NC Mountain and GA geographic populations were not significantly related based on the randomization test, the associated subpopulations, including nest groups (NC Mountain), that were related have some of the highest percent dyad relatedness values (midrange estimate: NC Mountain 50%, GA 39%; Table 3). Because most geographic populations were collected in a single trapping event, these nesting associations are a snapshot of the squirrels' nesting behavior and are not necessarily informative about nest-group structure and movements throughout the winter. Winter aggregations are not static; squirrels move opportunistically between nest sites and groups (Muul 1968; S. C. Loeb, United States Department of Agriculture–Forest Service, Clemson University, pers comm.).

Our results show patterns supporting those postulated by Layne and Raymond (1994) where kin interactions can be complex and nest groups often contain known 1st-order relatives or the same

squirrels over a span of months and years. Using marked litters they showed that 16% of nest groups contained at least one 1st-order dyad. Winterrowd et al. (2005) found 36% of adult-only nest groups contained a 1st-order dyad, whereas we found 86% of geographic populations contained a 1st-order dyad and 75% of known nest groups contained a 1st-order dyad. All 7 of our geographic populations consisted of adult-weight animals, suggesting our sample consisted of all adults. Any 1st-winter animals had attained adult weight. This differs from the populations sampled in other studies; 79% adult-only nest groups were seen in bimonthly surveys (Layne and Raymond 1994), and 58% adult-only nest groups were seen in spring (Winterrowd et al. 2005).

During the spring breeding season Winterrowd et al. (2005) found that adult-only nest groups showed low aggregation mean relatedness. However, mixed-age and family groups were highly related. The lack of close relatives in adult-only groups during spring could be caused by active aggression by females and avoidance of other squirrels (regardless of relatedness) during late pregnancy and lactation (Muul 1969). Alternatively, habitat disturbances such as squirrel removal or spring temperature-related reductions in aggregation size could be factors in the findings of Winterrowd et al. (2005). Given this, our results suggest that patterns of association among flying squirrels might depend on time of year and habitat constraints. Behavioral assays in captivity show that southern flying squirrels preferentially nest with kin when forming winter aggregations, and littermate siblings preferentially nest together as adults (Thorington 2008; K. K. Thorington, pers. obs.). Therefore, we think these squirrels show a marked preference for kin as winter nest mates (Thorington 2008). Both the seasonal selection of winter nest mates during the declining photoperiod in the fall and then the stability of groups throughout the winter season are probably mediated by kinship. Southern flying squirrels are familiar with their home range and its inhabitants (Sawyer and Rose 1985). Evidence supports an attempt to exclude unfamiliar squirrels by those animals using the same resources (Muul 1968).

The observed variation in relatedness of nest mates requires consideration of the potential benefits of nesting with kin in winter. Southern flying squirrels rely on the energetic advantages provided by abundant stored food, good nest sites, and communal nesting for winter survival (Muul 1968; Tompkins 2003). Examination of trapping data shows that distribution of squirrel populations often is patchy; in high-quality habitat they frequently occur in large groups or multiple groups, and they have multiple active nests (Doby 1984). Food storage by individuals, although not a communal activity, can increase the concentration of food resources available in the area. Because such food is not defended vigorously by the squirrels, it can benefit all animals relying on the food source. Southern flying squirrels are effective at locating stored food items. Despite the storer's advantage, squirrels are opportunistic in finding, stealing, and eating or stealing and recaching food items previously stored by other animals including nest mates (Winterrowd and Weigl 2006). Therefore, food energy stolen by related nest mates has the potential to facilitate winter survival by increasing heat available in the nest and also can increase inclusive fitness that transmits the individual's genes into the next generation through offspring of nest mates.

Given the potential benefits of aggregating with kin, why would southern flying squirrels sometimes nest with nonrelatives? We suggest that habitat constraints could drive this behavior. The distribution of southern flying squirrels is patchy between years, even in high-quality habitat (Doby 1984). The availability of storable food is especially important in determining the

presence of populations in marginal habitat such as that on the northern end of the range (Bowman et al. 2005), at higher altitudes, or in forest with few mast-producing trees. Therefore, habitat quality may influence the availability of kin as winter nest mates. The pattern of higher relatedness among winter nest mates in high-quality habitat shown here suggests that in stable habitat groups may persist longer and have a strongly kin-mediated population structure. When conditions are variable, such as those at the northern ends of the range or the elevational limits of hardwoods (i.e., the NC Mountain population), animals are likely to move or not survive the winter. In these circumstances kin might be scarce, requiring opportunistic choices for nest mates, and the thermoregulatory advantages of communal nesting could outweigh the costs of food losses to nonkin in these disturbed or marginal situations. Low relatedness may still generate a fitness benefit, in addition to the energetic advantages of group living, as long as it is greater than the background relatedness level (Kerth 2008; Kerth et al. 2002; West-Eberhard 1975). However, even in populations that are less related than would be expected by chance, such as the AL population, we still found approximately 33% of dyads within the nest group to be highly related.

Behaviors leading to kin-based winter aggregation should be explored in the context of fission–fusion dynamics seen in bats and other potential group structures as seen in other squirrels (Metheny et al. 2008a, 2008b; Sherman 1981). In related studies we found behavioral preferences for kin during aggregation formation and persistence experiments with known-relationship animals in captivity (Thorington 2008). In the field known-relationship animals should be followed year-round to determine relationships between neighbors and nest mates during the summer; the amount of fission and fusion among squirrel aggregations over the course of the winter and whether relatedness affects which squirrels form the core group in an aggregation; and what happens in low-mast years—is pilfering more or less prevalent and is pilfering behavior seasonally variable? We would predict that groups contain a kin-based core and that in colder weather less-related animals would be tolerated more if food conditions are good.

Acknowledgments

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