

MOVEMENT AND GENE FLOW OF NORTHERN FLYING SQUIRRELS ACROSS
AN INTERSTATE HIGHWAY

by

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ABSTRACT

Roads are a pervasive cause of habitat fragmentation around the world. Roads can present barriers to movement through direct mortality, behavioral avoidance, or by acting as an impassable physical object in the landscape. The barrier effect of roads has been demonstrated for species from multiple taxa. Species inhabiting the interior of forests may be particularly sensitive to roads because of their inability or disinclination to traverse gaps in forest cover. We combined telemetry and molecular genetic techniques to examine the effects of a high-speed, high-volume highway on the movement and population genetic structure of northern flying squirrels (*Glaucomys sabrinus*) in the Cascade Mountains of Washington, USA. During 2009 and 2010, we trapped and radio-tracked flying squirrels ($n = 17$) to gather data on movement within their home ranges and to detect movement across the highway. Additionally, we tested for effects of the highway on genetic variation in the study area using DNA extracted from cheek cells of 59 squirrels and genotyped at 11 microsatellite loci. Seven of the 17 radio-tracked squirrels crossed the highway at least once during their nightly movements. The width of the gap between forest edges across the highway appeared to negatively influence crossing rates and no crossings were observed at a site where the average gap width exceeded 80 meters. Genetic analysis provided no evidence that either geographic distance or the presence of the highway was associated with genetic differences between sites at the landscape scale. Results suggest that populations on either side of the highway are well connected demographically and genetically, and that connectivity can be maintained if gaps in forest canopy associated with the highway are kept to a width within the gliding range of flying squirrels.

INTRODUCTION

Habitat loss and fragmentation can have negative consequences for populations by decreasing their size and reducing immigration from other populations. Small populations inhabiting remnant habitat fragments face heightened extinction risk from environmental, demographic, and genetic effects. Environmental fluctuations and random catastrophes are more likely to threaten persistence in small populations (Gilpin and Soulé 1986; Lande 1993), and, when isolated, the “rescue effect” of immigration is less likely to save small populations from incipient extinction (Brown and Kodric-Brown 1977). Negative genetic consequences may interact with and exacerbate these demographic effects to further reduce persistence in populations that become small and isolated.

Three genetic issues arise as population size decreases. First, the tendency for genetic drift to erode genetic diversity increases (Frankel 1974). Second, inbreeding will become more common, unmasking deleterious recessive alleles in the population by increasing homozygosity (Crnokrak and Roff 1999; Hedrick and Kalinowski 2000; Ralls et al. 1988; Wright 1969). Third, deleterious mutations of mild effects are predicted to accumulate to the detriment of population fitness, potentially leading to a “mutational meltdown” (Gilligan et al. 1997; Kimura et al. 1963; Lande 1994; Lynch et al. 1995a, b).

Fragmentation and habitat loss often occur simultaneously, but in some cases barriers to the movement of individuals may divide populations without substantially reducing their total size. The genetic consequences of reduced gene flow *per se* among subpopulations are complex and highly dependent on the size of isolated subpopulations

and the rate of gene flow among them, but generally, reduction in fitness or persistence due to inbreeding depression, loss of genetic diversity, and mutation load is expected to be greater under conditions of highly restricted gene flow (Couvet 2002; Higgins and Lynch 2001; Jaquiere et al. 2009; Theodorou and Couvet 2006). Empirical studies comparing genetic structure between continuous and fragmented landscapes have generally found that inbreeding is more prevalent, genetic diversity is lower, and genetic differentiation is higher in fragmented landscapes (see review by Keyghobadi [2007]).

While it is generally accepted that fragmentation has mostly negative effects on populations, the magnitude of these effects depends critically on factors specific to the landscape and species in question. There may be, for example, thresholds in the distribution and abundance of suitable habitat below which a landscape is functionally disconnected for a species occupying that habitat (With and Crist 1995; With et al 1997). Species vary widely in their movement and dispersal ability through various types of land cover. Differences in body size, vagility, habitat preference, and morphology may all contribute to the variability in species' responses to habitat fragmentation (Lindenmayer and Lacy 1995; With and Crist 1995; Kerth and Melber 2009).

Roads are barriers to movement for many taxa, fragmenting formerly continuous populations into smaller, more isolated subpopulations. Roads may pose barriers to movements of individuals via direct mortality, behavioral avoidance, or by acting as an impassable physical obstacle in the landscape (Forman and Alexander 1998; Mader 1984). Forman and Alexander (1998) suggested that although an estimated one million vertebrates are killed on roads every day in the United States, the barrier effect of roads is

likely a more serious threat to most populations than is increased mortality associated with traffic. Mark-recapture studies and translocation experiments have shown that wider roads tend to inhibit movement of mammals more than narrow roads (Mader 1984; Oxley et al. 1974), although Swihart & Slade (1984) found that even very narrow roads (< 3 m) strongly inhibited movement of cotton rats (*Sigmodon hispidus*) and prairie voles (*Microtus ochrogaster*). Despite a demonstrated barrier effect of two-lane paved roads, McGregor et al (2008) found that traffic volume did not have a significant effect on crossing probability in eastern chipmunks (*Tamias striatus*) and white-footed mice (*Peromyscus leucopus*), suggesting that these species avoid the road itself (or the habitat gap), not necessarily just traffic. Evidence also suggests that body size, habitat specialization, and mobility may all influence whether a species' movement is inhibited by roads, with smaller, habitat interior-associated, and low-mobility species most affected (Laurance et al. 2004; Mader 1984; Oxley et al. 1974; Shepard et al. 2008).

Recent methodological advances are advancing our capability to correctly identify when potential barriers have negative effects on populations. Until the last decade, most studies of road barrier effects used direct observation of movements of a sample of individuals—for example, using mark-recapture, radio-telemetry, or global positioning system (GPS) collars. It has been suggested, however, that one to ten migrants per generation is sufficient to prevent loss of genetic diversity within subpopulations (Mills and Allendorf 1996). This amount of movement across a potential barrier could be very difficult to detect with the sample sizes and durations of most field studies. Thus, while

these methods have their advantages, it is difficult to detect the low rates of migration necessary to prevent negative genetic effects on fitness.

Molecular genetic techniques present a potential solution to this problem. Neutral genetic markers have shown great potential to uncover fine-scale population structure and are increasingly being used to detect population-level effects of habitat fragmentation on genetic diversity within and between populations (Keyghobadi 2007). Genetic techniques are effective tools for detecting barrier effects of anthropogenic habitat fragmentation and evaluating mitigation strategies because highly variable genetic markers such as microsatellites can be used to detect effects at recent temporal and fine spatial scales (Balkenhol and Waits 2009).

Although roads are relatively recent features of landscapes, and time is required to detect interrupted gene flow, many studies have shown that the barrier effect of roads are reflected in contemporary population genetic structure (Holderegger and Di Giulio 2010). For example, microsatellites revealed lower genetic diversity and higher differentiation among populations of agile frogs (*Rana dalmatina*) in France separated by a highway built only 20 years ago than among populations in an unfragmented area (Lesbarrères et al. 2006). Results such as these demonstrate that roads may very rapidly isolate populations of certain species. Road effects on either genetic diversity or genetic differentiation have been shown in insects (Holzhauer et al. 2006; Holzman et al. 2009; Keller and Largiader 2003; Vandergast et al. 2009), amphibians (Hitchings and Beebee 1997; Johansson et al. 2005; Marsh et al. 2008; Reh and Seitz 1990), a reptile (Clark et

al. 2010), and many species of mammals (Coulon et al. 2006; Epps et al. 2005; Gerlach and Musolf 2000; Kuehn et al. 2007; Perez-Espona et al. 2008; Riley et al. 2006).

Genetic tools alone, however, provide little information about the mechanisms responsible for the broad patterns observed in the genotypes. Combining traditional field-based techniques with molecular genetic tools can strengthen inferences about barrier effects and ensure that important patterns are not overlooked. Inferring restricted movement or dispersal capability from observed genetic differentiation may be incorrect (Booth et al. 2009). Behavioral dispersal may occur without consequent gene flow if, for example, dispersing animals encounter tightly defended territories upon arrival at their destination (Booth et al. 2009; Riley et al. 2006). Alternatively, inferring that animals move freely across a barrier based on a lack of differentiation could also be misleading, as genetic structure, especially in large populations, takes time to respond to interruptions in gene flow. Here, we measure both movement and genetic patterns directly to avoid these potential problems.

The northern flying squirrel (*Glaucomys sabrinus*) is an interesting focal species with which to explore the barrier effect of roads in forested landscapes because its locomotion is largely dependent on forest structure. Like other gliding mammals, northern flying squirrels move primarily by gliding from tree to tree. Horizontal gliding distance is limited by their anatomy, which determines their glide ratio, and by the height from which they initiate a glide. Maximum glide distance recorded for *G. sabrinus* is 65 m (Scheibe et al. 2006) and more typical distances are 20–40 m (Vernes 2001). While the vast majority of their movement is through the arboreal canopy (Carey 2000a), flying

squirrels spend more time on the ground than many other gliding mammals, as they feed primarily on below-ground fruiting bodies of mycorrhizal fungi (e.g., *Rhizopogon spp.*, *Gautieria spp.*). Their movement on the ground, however, is slow and clumsy (Maser et al. 1986; Wells-Gosling and Heaney 1984). Crossing open ground may, therefore, increase predation risk. For these reasons wide gaps in forest canopy, such as those associated with large roads, may be barriers to movement for flying squirrels.

Weigl et al (2002), studying a population of northern flying squirrels in the southernmost extension of their range in North Carolina divided by a two-lane highway, did not observe crossings or crossing attempts by any of ten radio-tracked adults over a period of two years. The highway in this study averaged only 38 m from forest edge to forest edge—well within the gliding range of adult flying squirrels. This suggests flying squirrels may avoid crossing roads for reasons other than physical limitations.

Dispersal and movement habits with regard to habitat configuration are poorly studied in northern flying squirrels, but have been studied in several similar gliding mammals. Juvenile Siberian flying squirrels (*Pteromys volans*), which have gliding abilities very similar to northern flying squirrels (Asari et al. 2007; Vernes 2001), tended to disperse through preferred habitat; open areas that could not be crossed in a single glide were almost always avoided (Selonen and Hanski 2004). In a related study, one adult male was observed to cross a field 70 m wide in a single glide several times, but only one female crossed a gap wider than 50 m (Selonen and Hanski 2003). In Victoria, Australia, road crossing by squirrel gliders (*Petaurus norfolcensis*) was inhibited, though

not precluded, by wide gaps (i.e., 50+ m) in tree canopy created by roads and power lines (van der Ree 2006; van der Ree et al. 2010).

Using a combination of radio-telemetry and microsatellite DNA analysis, we aim to describe the effect of Interstate 90 (I-90), a major east-west travel corridor in western Washington, USA, on the movement and gene flow of northern flying squirrels— a species of conservation interest in the Cascade Mountains of Washington (Lehmkuhl et al. 2006). A 15-mile section of I-90 bisecting the Cascades in West-central Washington will be widened and retrofitted with a variety of wildlife crossing structures over the next decade. In cooperation with Washington State Department of Transportation (WSDOT), several agencies and university researchers are collaborating to monitor the effects of the connectivity enhancement measures for a suite of focal species with a range of habitat associations and ecological limitations (Noss 1999). This list of species was systematically chosen based on mobility, representation of community types, and commonness, and includes elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), black bear (*Ursus americanus*), cougar (*Puma concolor*), bobcat (*Lynx rufus*), American marten (*Martes americana*), pika (*Ochotona princeps*), northern flying squirrel, and several species of murid rodents, reptiles, and amphibians (Clevenger et al. 2008). By monitoring these species before and after mitigation, researchers and managers hope to get a more comprehensive picture of highway effects and responses to connectivity mitigation efforts. Thus, an additional objective of this study is to establish baseline data that can later be used to compare connectivity of northern flying squirrel populations across I-90 before and after mitigation measures are implemented.

MATERIALS AND METHODS

Study Site

We conducted this study along a 24 km segment of I-90 on the east slope of the Cascade Range from just east of Snoqualmie Pass to the town of Easton, Washington (Figure 1). Vegetation consisted of mixed coniferous forest dominated by Western hemlock (*Tsuga heterophylla*) and Douglas-fir (*Pseudotsuga menziesii*). Riparian areas adjacent to the Yakima River and its tributaries also supported western Red-cedar (*Thuja plicata*) and black cottonwood (*Populus trichocarpa*). The rain-shadow effect of the Cascade Mountains caused a prominent west-to-east moisture gradient from 100+ inches of annual precipitation near Snoqualmie Pass to around 50 inches annually at the town of Easton (USFS 1997). Stand age varied considerably over the study area and ranged from recent clear cuts to late-successional stands in excess of 600 years old.

Lower elevations in the Yakima River valley consisted of a mix of National Forest lands managed primarily for recreation and wildlife habitat, and privately owned recreational, residential, and commercial timberland. Wilderness areas exist several miles to the north (Alpine Lakes Wilderness) and south (Norse Peaks Wilderness).

Interstate 90 (I-90), a primary focus of this study, is a high speed, high volume roadway and the largest of four major travel corridors across the Cascade Mountains in Washington. Traffic volume averaged 28,000 vehicles per day from 2004 to 2007 (WSDOT 2007). While lands surrounding I-90 comprise a zone of intense anthropogenic disturbance and fragmentation (e.g., I-90, two high-voltage electric transmission lines, a

railroad, and checkerboard land ownership with contrasting management goals), this corridor represents a relatively narrow gap in otherwise largely intact natural montane forest habitat stretching north and south along the Cascade divide. National Forest lands are administered by Okanagan-Wenatchee National Forest (OWNF) under the USFS Snoqualmie Pass Area Adaptive Management Plan, which emphasizes management for late successional (old-growth) forest habitat and wildlife connectivity. This area has been identified by OWNF as a critical connectivity zone for wildlife in the Cascade Mountains.

Sampling Methods

Animal Capture

We trapped squirrels in June and July of 2009 and 2010. We attempted to locate trap sites (Figure 1) in suitable habitat within one flying squirrel home-range diameter of the highway in an effort to sample only squirrels with home ranges adjacent to the highway. We located trap sites in pairs across the highway from each other so we could potentially detect crossings by recapturing marked individuals (see below) at the trap site opposite their capture. We placed trap stations approximately 30 m apart along lines oriented parallel to the highway. Trap sets were often oddly shaped to fit within stands, but generally comprised 24–36 trap stations and covered approximately 1.8 to 2.7 ha. At each trap station, we placed one 41x13x13 cm trap (Tomahawk Model 201, Tomahawk, WI) on the ground and fixed one to the bole of a nearby tree at chest- to head-height (1.25–2 m above the ground). We covered traps with tight fitting wax-coated cardboard

boxes, and placed smaller cardboard nest boxes with polyester bedding inside traps to protect trapped animals from exposure. Finally, we covered traps with natural debris to make them less conspicuous. We opened traps in the evening and checked them early the following morning, closing traps during the day to reduce captures of non-target species. We baited traps with a mixture of peanut butter, rolled oats, and molasses and changed or added bait daily.

We weighed, sexed, and assessed all trapped flying squirrels with regard to reproductive status. Squirrels that were not radio-collared (see below) were marked with passive integrated transponder (PIT) tags so that recaptured individuals could be identified. After processing, we released squirrels at their location of capture and monitored them briefly to ensure they had not been injured during handling.

Movement and home range

To examine the effect of I-90 on the movement of individual squirrels, we used radio-telemetry to track nightly movements and describe the home ranges of a subset of captured squirrels. We fitted captured flying squirrels with a 4 g VHF transmitter (Model PD-2C, Holohil Systems Ltd., Carp, Ontario, Canada) affixed around the neck with 30 lb.-test braided steel fishing line and padded with flexible PVC tubing. To ensure gliding capability was not impeded by the transmitters, we only collared squirrels ≥ 120 g in order that the combined weight of the transmitter and collar would not exceed a conservative 4% of the body mass of the animals (Cochran 1980).

We used flexible-element handheld antennae (Telonics, Inc., Mesa, AZ, USA), to closely approach and record locations of tracked squirrels at one-hour intervals during

each tracking session. Locations were recorded with handheld global positioning system (GPS) units. Observers estimated and recorded the accuracy of each location as 1 (visual confirmation or likely tree location of the squirrel), 2 (within 20 m of the squirrel), 3 (within 40 m of the squirrel), or 4 (poor quality point, usually when the observer could not physically approach the squirrel or obtain a strong signal). Locations with accuracies of 4 were omitted from analysis.

We observed movements from late June through late August in 2009 and 2010, tracking each squirrel one to three nights per week; the resulting data consisted of several bursts of 1 hr-spaced locations clustered temporally by individual tracking sessions but spread evenly over the 2-3 month monitoring period.

Molecular Genetics

Samples of epithelial cells were obtained from all captured flying squirrels by swabbing the inside of the cheek with cotton and synthetic swabs. Samples were stored with silica desiccant until extraction.

We isolated genomic DNA from cheek swabs (cotton and/or synthetic) using the Qiagen's Investigator Kit. Eleven polymorphic microsatellite loci were chosen for the purpose of genotyping (Table 1 in Appendix A). To view PCR amplicons we used fluorescently labeled universal M13 primer, with an attached M13 sequence at the 5' end of the forward primer. All loci shared the same PCR chemistry which consisted of 2 μ L of 5X MyTaq RXN Buffer, 1 μ M of each primer, 0.5 Unit of MyTaq™ HS DNA Polymerase, ~50ng of DNA and enough water for a final volume of 10 μ L (Bioline). Similarly, all 11 loci share the same thermoprofile which consisted of one activation step

at 95°C for 1 min followed by 30 cycles (95°C for 15 s, 55°C for 15s and 72°C for 10 s). To incorporate the fluorescently labeled universal M13 primer, 10 additional cycles were performed (95°C for 15 s, 53°C for 15s and 72°C for 10 s). PCR amplicons were visualized using the 3100-*Avant* Genetic Analyzer and scored with GeneMapper v3.5 (Applied Biosystems).

Analysis Methods

Movement and Home Range

We converted pairs of sequential locations for a given squirrel into a number of movement vectors. These vectors were simply straight-line movement segments representing simplifications of typical movements of squirrels during an hour or so of nightly activity. Observers were often unable to find a squirrel or record an accurate location at exact 1-hour intervals so we relaxed this constraint slightly and used only locations recorded between 50 and 120 minutes apart in the analysis. We used a Monte Carlo randomization procedure in R (R Core Development Team 2010) to test if these movement vectors crossed the highway less frequently than could be ascribed to chance. This procedure involved repeatedly a) selecting a random sample (without replacement) of known locations for a given squirrel equal to the number of movement vectors observed for that squirrel, b) randomly assigning distances (sampled with replacement from observed vectors) and bearings (sampled from a uniform distribution from 1–360) to those points to simulate random movement vectors, and c) recording a count of the number of random vectors that would have crossed the highway. We determined p-values

by calculating the proportion of 10 000 randomizations with crossing counts \leq the observed number of crossings.

We determined home ranges by constructing simple minimum convex polygons in ArcGIS 10 (Esri, Redlands, CA, USA). I only calculated home range sizes of squirrels for which we had ≥ 30 locations.

Molecular Genetics

We screened genotypes for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium using the computer program GENEPOP 4.1 (Raymond and Rousset 1995; Rousset 2008), assigning α levels using sequential Bonferroni correction for multiple comparisons (Rice 1989). We also used GENEPOP to estimate allele frequency-based fixation indices (F_{ST}) between all possible pairs of trapping sites using Weir and Cockerham's (1984) estimator θ , and pairwise individual genetic distances among all individuals using Rousset's (2000) \hat{a} , which is somewhat analogous to $F_{ST}/(1 - F_{ST})$ (see Rousset 1997) for assessing isolation by distance of individual squirrels.

We employed Mantel tests and partial Mantel tests (Mantel 1967; Smouse et al. 1986) to test for effects of geographic distance and the highway on genetic differentiation at both the site level (pairwise F_{ST}) and the individual level (\hat{a}). Geographic coordinates of individual animals were determined in one of two ways: for animals not radio-tracked, we used the location of capture; for radio-tracked animals, we used the center of their home range, defined by the mean Universal Transect Mercator (UTM) easting and mean UTM northing of all recorded locations. The geographic coordinates of a site were subsequently established by taking the means of the coordinates of all individuals

sampled at that site. We used the natural logarithm of the Euclidean distance (in meters) between populations or individuals in evaluating isolation by distance. Mantel tests were performed in the R package ECODIST v 1.2.3 (Goslee and Urban 2010) and significance was determined with 100 000 randomizations.

We also used the Bayesian population assignment software STRUCTURE v.2.3 (Pritchard et al 2000) to infer the most likely number of populations (K) in the study area and to examine the relationships between the inferred populations and landscape features that might affect gene flow. The most recent version of STRUCTURE allows user-defined “populations” to be associated with each individual, thereby improving the program’s ability to correctly assign individuals to groups when genetic structure is weak or when samples are clumped in space (Hubisz et al 2009). We chose to use this feature because of the limited geographic extent of our samples and their clumped distribution. We used the model parameters recommended in the software documentation (i.e., the admixture model with correlated gene frequencies, α inferred from the data, $\lambda=1$, a burn-in period of 10 000 iterations, and 10 000 iterations of the Markov chain). We used the methods described in Evanno et al. (2005) to infer the most likely value of K based on 5 independent runs at each value of K from 1 to 6 (the maximum value being the number of sites from which the samples were collected).

Finally, we used the program BARRIER v.2.2 (Manni et al. 2004) to identify the most likely location of a gene flow barrier. BARRIER uses Monmonier’s algorithm to locate discontinuities in gene flow based on the locations of individuals and the magnitude of pairwise individual genetic distances (\hat{a}).

RESULTS

Movement and Home Range

We deployed radio collars on 11 squirrels in 2009 and 10 squirrels in 2010. Over both years, three collars slipped off or were removed by squirrels and were recovered and re-deployed on other individuals and one collared squirrel ceased movement after several days and was presumed to have died or slipped its collar. In total, we tracked 17 squirrels long enough to collect usable movement data and recorded 548 locations. Table 1 summarizes the telemetry data collected during the study.

Home ranges of squirrels with ≥ 30 locations ($n = 11$) ranged from 0.85 to 67.60 ha. Only three females fit this sample size criterion, but the range of home range sizes of these females (0.85–8.93 ha, mean = 3.88 ha) did not overlap the range of home range sizes of the males (10.67–67.60 ha, mean = 24.99 ha). Maps of simple minimum convex polygon home ranges are shown in Figures 2–4.

Squirrels successfully crossed the highway during both years and at three out of the four sites—Bonnie Creek, Toll Creek, and Easton Island. No crossings were detected at Easton Hill. Figure 5 shows all detected crossings at each site. Seven squirrels (~ 41%) were observed to have crossed the highway at least once. Among squirrels that were observed to have crossed at least once, crossing rates (the proportion of observed vectors that crossed the highway) ranged from 0.06 to 0.46. Crossing rates did not differ between males and females (exact Wilcoxon rank sum test, $W = 22.5$, $p = 0.27$). Among the four sites, crossing rate was highest at Easton Island (0.18), where the treed median separating

the east- and west-bound lanes meant that squirrels only had to cross two lanes of traffic, and lowest at Easton Hill (0.0), which had the greatest width between forest edges (Figure 6).

Results of the randomization tests are reported in Table 1. A total of 333 movement vectors were used in the analysis. Highway crossings comprised about 10% of recorded movements in 2009, 9% of movements in 2010, and 9% of movements over both years combined. When all squirrels were pooled over one or both years, results indicate that highway crossings occurred significantly less frequently than would be expected if they did not avoid crossing the highway (Monte Carlo $p < 0.0001$, all squirrels combined). The highway appeared to reduce crossings by approximately 48% when all vectors were pooled for analysis (expected crossings = 59.23, observed crossings = 31, 95% confidence interval from 32.6–57.5% reduction).

Molecular Genetics

A total of 59 individuals were genotyped at 11 loci. Using exact G tests implemented in GENEPOP, seven of 55 pairs of loci showed potential linkage ($p < 0.05$) but there was no evidence for gametic disequilibrium among any pairs of loci after sequential Bonferroni correction. The average number of alleles per locus was 7.73 (range 5–13). The average observed heterozygosity was 0.62. Number of alleles and observed and expected heterozygosities for all loci are tabulated in Appendix A. After sequential Bonferroni correction, Bonnie Creek South was the only site that deviated

significantly from Hardy-Weinberg equilibrium (Fisher's method, $\chi^2 = 42.905$, d.f. = 20, $p = 0.0021$).

We found statistically significant genetic structure between all but three pairs of sites (Table 2). Pairwise F_{ST} among sites on the same side of the highway (mean = 0.050, range 0.014–0.77) was very similar to pairwise F_{ST} among sites on opposite sides of the highway (mean = 0.051, range 0.011–0.91). Global F_{ST} was 0.051. There was no evidence for a correlation between pairwise F_{ST} and geographic distance (Mantel's $r = -0.18$, $p = 0.77$) or between pairwise F_{ST} and highway presence (Mantel's $r = 0.026$, $p = 0.39$).

Because of the close proximity of sampled individuals from the same trap site, we modified the individual-based analysis to exclude comparisons between individuals below a threshold geographic distance. We chose to do this for three reasons. First, the relationship between geographic distance and genetic distance is thought to deteriorate at distances that are below the dispersal distance of individuals, σ (Rousset 1997, 2000), and the size of our trap sets resulted in clusters of individuals far below this threshold distance from one another. Second, relatedness analysis performed in the program MLRELATE (Kalinowski et al. 2006) indicated that individuals within sites were highly related (average relatedness within sites, $\bar{r}_{within} = 0.13$, average relatedness overall, $\bar{r}_{overall} = 0.06$). Pairwise comparisons between members of family groups were not desired; we were interested in whether the highway was associated with genetic differences at the population scale rather than how it influenced the distribution of family groups at a very fine spatial scale. Finally, estimated geographic distances between individuals within

sites were unreliable because home ranges overlapped considerably and any error associated with these estimates would be magnified by the log transformation of distance. We therefore chose to exclude comparisons of individuals sampled less than 500 m apart. This excludes most comparisons within trapping sites and, as an approximation of σ , is in close agreement with the predicted mean dispersal distance of 430 m reported in D'Eon (2002) for northern flying squirrels. This estimate of σ may be low given the distribution of 50–120 minute movement lengths directly observed; although 96% of these movements were less than 500 m, squirrels were observed moving as far as 974 m in less than two hours.

After exclusion of comparisons between individuals below the 500 m threshold, the remaining distance dataset included 79% of the original distance dataset. A simple linear regression test indicated that neither geographic distance nor the barrier effect of the highway was significantly correlated with genetic distance ($t = 0.441$, $df = 1454$, $p = 0.659$ for geographic distance and $t = -0.003$, $df = 1454$, $p = 0.998$ for barrier effect). Simple linear regression should not be used to test significance of these correlations because non-independence among observations will always result in artificially small standard errors and, subsequently, inappropriately small p-values. Therefore, large p-values under the simple linear regression model are a strong indication of a lack of a relationship between the explanatory variables—geographic distance and the highway—and genetic distances between individuals.

The program BARRIER identified the most likely barrier occurring through the Easton Hill North site, perpendicular to the highway. The location of the inferred barrier did not correlate with any landscape feature that we could identify.

Using the ΔK method of Evanno et al. (2005), STRUCTURE identified $K = 4$ as the most likely number of groups, but members of these inferred groups were geographically mixed. Thus, while genetic structure was evident among the sampled squirrels, patterns consistent with geographically distinct groups did not emerge. Relatedness analysis in MLRELATE gave results consistent with this. Relatedness coefficients ($r \geq 0.25$) (consistent with half-sibling relationship) were found between squirrels in 11 of the 15 possible site pairs, including 6 of 9 possible across-highway site pairs.

DISCUSSION

While the highway appeared to filter the movement of northern flying squirrels, it was not an absolute barrier to movement; almost half of the squirrels we tracked (41%) were observed to have crossed the highway at least once. Two squirrels—031 and 120—were only detected once on the opposite side of the highway from their site of capture. For these individuals, habitat on the opposite side of the highway may not represent part of their home range as it is usually defined. Instead, these observed crossings may represent extraterritorial “prospecting forays,” for example to seek mates (both were males). Five other squirrels crossed the highway on a more regular basis—some almost every night they were tracked. These squirrels often followed similar routes on both sides of the highway and frequented similar places, indicating that their true home range included territory on both sides of the highway. Thus, the result of the randomization test should be interpreted as an average effect on the squirrels observed in this study and not directly applicable to any specific squirrel.

Some variation in crossing behavior may be attributable to among-site differences in the barrier itself. Canopy gap width and crossing behavior varied predictably among the four tracking sites (Figure 6). At Toll Creek and Easton Island, where canopy gap was less than 50 m in places, five of nine tracked squirrels were observed crossing, often multiple times per night. In contrast, at the top of Easton Hill, the minimum canopy gap exceeded 65 m (and was > 80 m along most of the length of the site), tall conifers were set back from the forest edge, and an elevational gradient would have made gliding from south to north difficult, if not impossible. The canopy gap at Bonnie Creek was also wide

(> 60 m) but mature, tall conifers approached the very edge of the highway verges on either side. Finer scale movement data and the inclusion of several more sites along the highway would be necessary to quantitatively address how highway characteristics and gap width influence crossing behavior, but our results are consistent with the hypothesis that wide canopy gaps inhibit crossing by flying squirrels.

Given the observed rates of movement across the highway, one would expect that populations on either side are well connected both demographically and genetically. The results of the molecular genetic analysis generally corroborate the findings of the telemetry study. We were not able to detect a significant effect of the highway on genetic distances between squirrels at the landscape scale.

While some caution is advised when interpreting the results of our genetic analysis as an indicator of current rates of gene flow—recent anthropogenic barriers to dispersal may be difficult to detect in species with large population sizes (e.g. Gauffre et al. 2008)—simulations have shown partial Mantel tests to be sensitive to even very recent barriers; significant positive values of Mantel's r can be expected in 1–15 generations after establishment of a complete barrier (Landguth et al 2010). I-90 has existed in its present form for 50–60 years, which corresponds to a sufficient number of generations that a substantial barrier effect, if present, should have been detected. Furthermore, our observation of movements across the highway provides strong evidence that the highway is not a dispersal barrier. Although the highway reduced the rate of crossing events significantly, more than 40% of tracked squirrels were observed crossing at least once

and it is reasonable to expect that the cumulative number of individuals moving across the highway is more than sufficient to prevent genetic differentiation.

Estimates of population differentiation among sites, however, were unexpectedly high for a vagile, non-territorial rodent given the geographic distances under consideration (Table 2). The extent of the study area was only about 8 kilometers and distances between the centers of sites ranged from 0.35–7.89 km (Table 2). For a species that is likely able to travel a kilometer in an hour (our longest recorded movement was 0.97 km in 83 minutes, and squirrels were frequently observed moving faster than that), one would expect dispersal distances that approach the extent of the area we studied. In light of the finding that neither geographic distance nor the highway had a detectable effect on genetic distances between squirrels, the high degree of differentiation among sites suggests that gene flow may be limited by philopatry rather than dispersal limitations imposed by the landscape. This was consistent with the results of the relatedness analysis, which indicated a high number of probable parent-offspring, full sibling, and half-sibling relationships within sites. The scale at which we sampled squirrels at each site—trap sets were much smaller than the average home range of our radio-tracked squirrels—gave us a group of individuals that more closely represented a family group than a random sample from the patch-level population, which is the unit we were interested in sampling for these comparisons. Estimates of F_{ST} were therefore more descriptive of differences between family groups than differences between randomly mating populations. Indeed, 57% of the variation in F_{ST} among pairs of sites was explained by mean within-site relatedness (Figure 8).

A review of empirical studies of the genetic effects of roads suggested that roads act as incomplete barriers to movement and gene flow for many species (Holderegger and Di Giulio 2010). The degree to which roads and their associated barrier effects impact populations depends on both the magnitude and the mechanism of the barrier effect. Road barrier effects can affect the persistence of wildlife population in three main ways: 1) traffic mortality during crossing attempts, 2) blocked access to resources, and 3) population subdivision (Jaeger et al. 2005). We did not observe any road-killed flying squirrels during this study, nor did any of our collared squirrels succumb to collisions with vehicles. Likewise, a search of roadkill databases did not turn up a single reference to flying squirrels found killed on roads despite an abundance of accounts of similarly-sized mammals, including tree squirrels and chipmunks. Flying squirrels do not migrate seasonally and are found at relatively high densities, so access to important habitat or mates would not seem to be an issue for those squirrels that did not cross. Finally, observed rates of movement across the highway and the genetic analysis indicate that population subdivision resulting from the barrier effect of the highway is not a problem currently facing this population.

Nocturnal species such as the northern flying squirrel may be less susceptible to barrier effects of roads because many of the risks associated with crossing are attributable to traffic rather than the road surface or the gap in habitat, *per se*. Traffic volume typically exhibits large and predictable fluctuations over the course of a day, dropping considerably after the commuting period in the evening and remaining fairly low until the morning commute. This period of low traffic volume corresponds well with the period of

activity for nocturnal animals, thus reducing their exposure to the risk and noise associated with traffic. Our observations in the field and data collected by WSDOT (Figure 9) suggest that even a high-volume highway such as I-90, within 60 miles of a major urban center, can experience dramatic drops in traffic volume at night. These predictable low traffic periods may be important for maintaining permeability of highways to wildlife given the number of species that are nocturnal or have some period of activity during the night.

Gap width—specifically the distance between tall trees on the verges of the highway—may affect the permeability of the highway to northern flying squirrels. Although we were unable to directly observe any crossings in this study, the general locations of the majority of crossings led us to believe they were accomplished by gliding. This was also supported by the fact that the single site at which no crossings were observed was the site with the greatest distance between trees on the verges and an elevational difference that would have likely precluded gliding from south to north. US Forest Service researchers studying wildlife connectivity across I-90 in our study area in the late 1990s conducted extensive monitoring of culverts to measure passage of small mammals; flying squirrels, though frequently detected by remote cameras in the area, were never detected crossing through culverts (Singleton and Lehmkuhl 2000). More recent remote camera monitoring efforts by the Western Transportation Institute (R. Long, Western Transportation Institute, personal communication) have also failed to detect flying squirrels crossing beneath I-90 via culverts. Even in the event that some crossings are accomplished by running across the road surface, these types of crossings

would be substantially more dangerous than gliding, and fewer successful crossings of this type would be expected.

In studies of other gliding mammals, gap width has been shown to influence the probability of successful crossings. Van der Ree (2006) compared crossing rates of nine radio-collared squirrel gliders (*Petaurus norfolcensis*) along a highway with a 12.2-m gap in canopy and a larger freeway with a gap of 55 m. All individuals at the highway site crossed the road on a regular basis, while two of the three individuals at the freeway site were never observed to have crossed, and the third crossed only infrequently. In a larger study of *P. norfolcensis* involving 47 radio-collared individuals, crossing rates were compared among three types of sites: control sites with canopy gaps < 9 m, freeway sites with trees in the median and canopy gaps of 5–13 m, and freeway sites without trees in the median and canopy gaps of 50–64 m. The proportions of gliders that made crossings were similar between control sites (77%) and freeway sites (67%) with trees in the median, but only 6% of gliders crossed at the freeway sites without trees in the median (van der Ree et al. 2010). Mahogany gliders (*Petaurus gracilis*) in Queensland, Australia crossed minor power line corridors and narrow roads with canopy gaps ranging from 8.7 to 27.9 m without apparent restriction, but only three of six gliders were observed on opposite sides of a highway (35.8 m wide) or a large power line corridor (31.5 m wide) and crossings of these gaps occurred less frequently than would be expected if the barriers had no effect on movement (Asari et al. 2010).

Our results suggest that connectivity across even high-volume highways can be maintained where there are gaps between tall trees across the roadway that do not exceed

the gliding ability of northern flying squirrels. Flying squirrels are clearly willing to cross the highway where opportunities exist. This finding is particularly germane in light of current transportation infrastructure trends. While construction of new roads in the United States has leveled off since the 1980s, traffic volumes continue to increase. In response, new lanes are added to existing roads to accommodate the added volume. Existing roads may thus become increasingly impermeable to wildlife. For motorists' safety, high-speed roadways are built to specifications that define a buffer, known as a clear zone, between traffic lanes and large trees on the verge. Adding new lanes to existing highways may therefore entail removal of trees and result in a widening of the canopy gap.

Where gaps in tree canopies exceed the gliding ability of flying squirrels, or where the addition of lanes will result in such gaps, appropriate mitigation measures for flying squirrels might include retention of tall trees at intervals on opposite sides of the highway, retaining or planting trees in the median where possible, or construction of specially-designed crossing poles. The latter strategy, which involves the placement of tall poles with horizontal launching platforms at the top on opposite sides of a road, has been used with success in aiding road crossings by endangered Australian squirrel gliders (*Petaurus norfolcensis*) in Victoria, Australia (Ball and Goldingay 2008) and endangered Carolina northern flying squirrels (*Glaucomys sabrinus coloratus*) in North Carolina, USA (Anonymous 2010). Voluntary use of the poles by animals has been documented in both cases. Fortunately, these mitigation measures are relatively simple and inexpensive compared to conventional wildlife crossing structures such as overpasses or underpasses.

The results of this study have direct applicability to current management issues in our study area. Highway construction beginning in 2010 will address deteriorating infrastructure and increasing traffic volume over this section of I-90. Traffic volume is increasing by an average of 2.1% per year and is projected to reach over 41,000 vehicles per day by 2030 (WSDOT 2008). Over the duration of the multi-phase construction project a new lane will be added in either direction, potentially widening canopy gaps in places that are currently traversable by flying squirrels. WSDOT plans to incorporate measures to increase connectivity for wildlife at 14 Connectivity Enhancement Areas (CEAs) along the stretch slated for improvements. Mitigation measures will include several wildlife crossing structures as well as fencing aimed at reducing wildlife-vehicle collisions. To specifically address northern flying squirrels we recommend that WSDOT consider the mitigation strategies mentioned above in addition to those currently planned.

TABLES

Site	Squirrel (sex)	Movement vectors	Tracking Nights	Observed Crossings	Expected Crossings	Crossing Rate	Monte Carlo <i>p</i> -value
Bonnie Creek	179 (F)	5	11	0	0.66	0	0.4942
	211 (F)	5	5	0	0.53	0	0.5697
	120 (M)	17	12	1	3.32	0.06	0.1273
	272 (M)	16	11	4	3.05	0.25	0.8261
Toll Creek	091 (M)	6	11	0	0.43	0	0.6452
	239 (M)	14	11	0	1.96	0	0.1187
	060 (M)	16	16	1	2.76	0.06	0.2057
	031 (M)	16	19	2	2.21	0.13	0.6201
	300 (F)	6	7	2	1.38	0.33	0.8623
Easton Hill	640 (M)	28	10	0	4.45	0	0.0065
	539 (F)	29	9	0	4.12	0	0.0114
	520 (M)	30	10	0	4.97	0	0.0038
	680 (F)	30	8	0	3.78	0	0.0188
Easton Island	818 (M)	15	4	0	2.98	0	0.0362
	178 (F)	32	10	0	6.47	0	0.0012
	498 (M)	33	10	5	8.32	0.15	0.1236
	739 (M)	35	7	16	7.64	0.46	0.9999
Pooled	2009	101	103	10	16.40	0.10	0.0493
	2010	232	68	21	42.81	0.09	<0.0001
	2009 and 2010	333	171	31	59.23	0.09	<0.0001

Table 1. Summary of radio-telemetry results and movement vector analysis. Expected crossings is the mean of the randomization distribution.

<i>Site Pair</i>	r_1	r_2	$r_{between}$	<i>Distance (km)</i>	F_{ST}
BCN-BCS	0.14	0.22	0.05	0.35	0.065**
BCN-TCN	0.14	0.11	0.07	2.49	0.014 (NS)
BCN-TCS	0.14	0.12	0.05	2.81	0.063*
BCN-EHN	0.14	0.17	0.08	7.18	0.056*
BCN-EHS	0.14	0.08	0.07	7.89	0.011 (NS)
BCS-TCN	0.22	0.11	0.05	2.26	0.072***
BCS-TCS	0.22	0.12	0.03	2.54	0.077***
BCS-EHN	0.22	0.17	0.03	6.93	0.091***
BCS-EHS	0.22	0.08	0.06	7.64	0.057**
TCN-TCS	0.11	0.12	0.05	0.48	0.060***
TCN-EHN	0.11	0.17	0.05	4.69	0.058***
TCN-EHS	0.11	0.08	0.06	5.40	0.022 (NS)
TCS-EHN	0.12	0.17	0.07	4.38	0.034**
TCS-EHS	0.12	0.08	0.05	5.10	0.042***
EHN-EHS	0.17	0.08	0.05	0.72	0.044**

Table 2. Pairwise geographic distances and $F_{ST}(\theta)$, Weir & Cockerham 1984) between sites. NS = not significant, * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.0001$ (after sequential Bonferroni correction). Average relatedness is reported within each site (r_1 and r_2) and between sites ($r_{between}$). Bold values indicate average coefficients of relatedness consistent with first-cousin relationships.

FIGURES

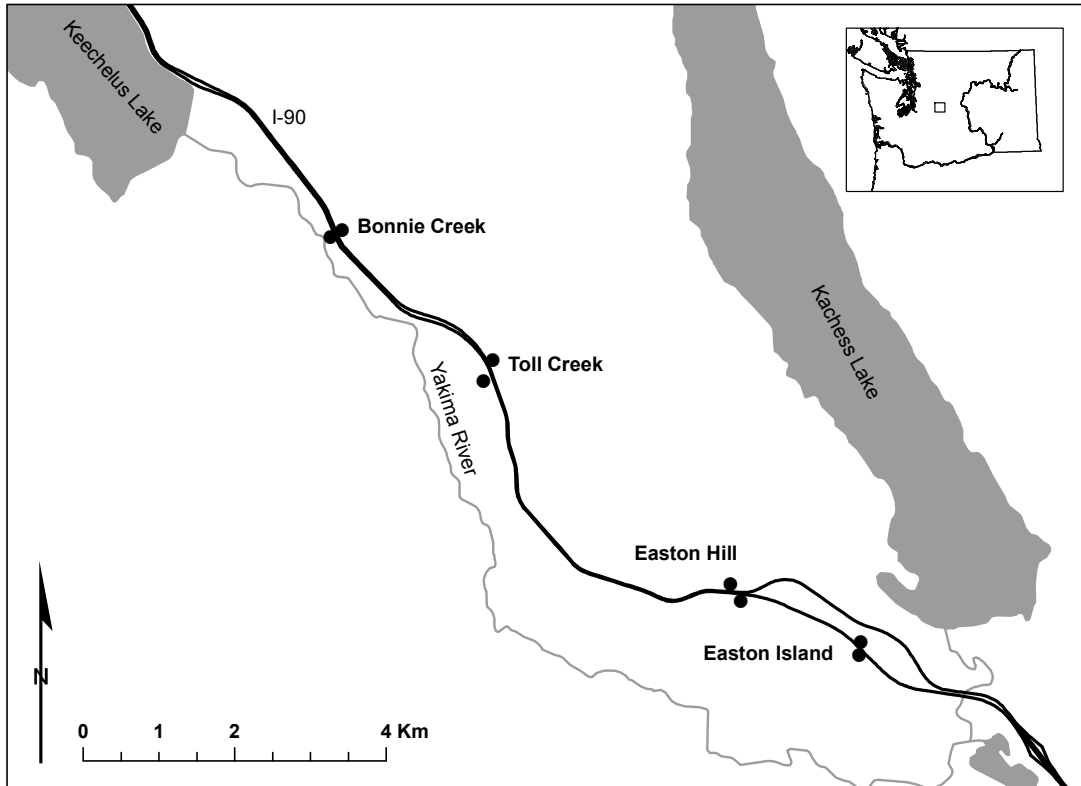


Figure 1. Map of study area showing locations of all trapping sites. Sites are referred to in the text and tables using the site names indicated on the map and either “North” or “South” to indicate their position relative to the highway. Animals captured at Easton Island South were combined with those captured at Easton Hill South for genetic analysis (all referred to as Easton Hill South) because of small sample sizes at each of these sites. Tests for departures from Hardy-Weinberg equilibrium supported this grouping.

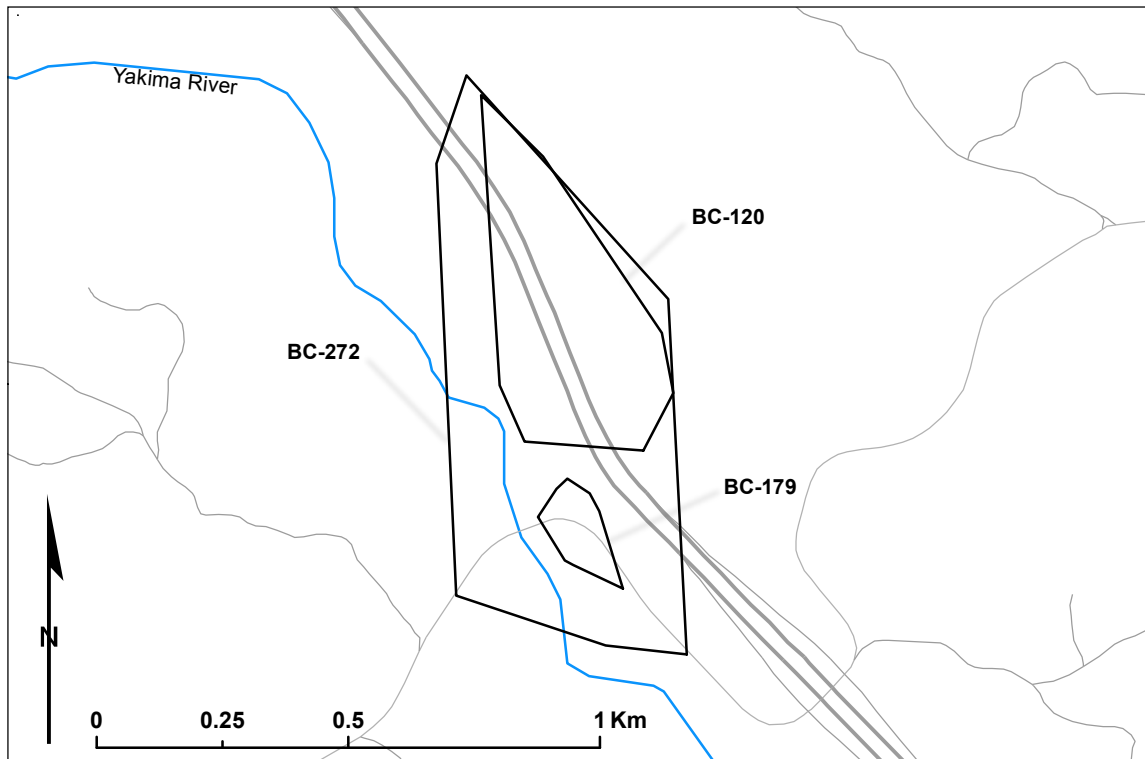


Figure 2. Simple minimum convex polygon home ranges of squirrels tracked at Bonnie Creek. The thick double line shows the position of the east- and west-bound lanes of I-90. Thin gray lines indicate on- and off-ramps as well as smaller paved and unpaved roads.

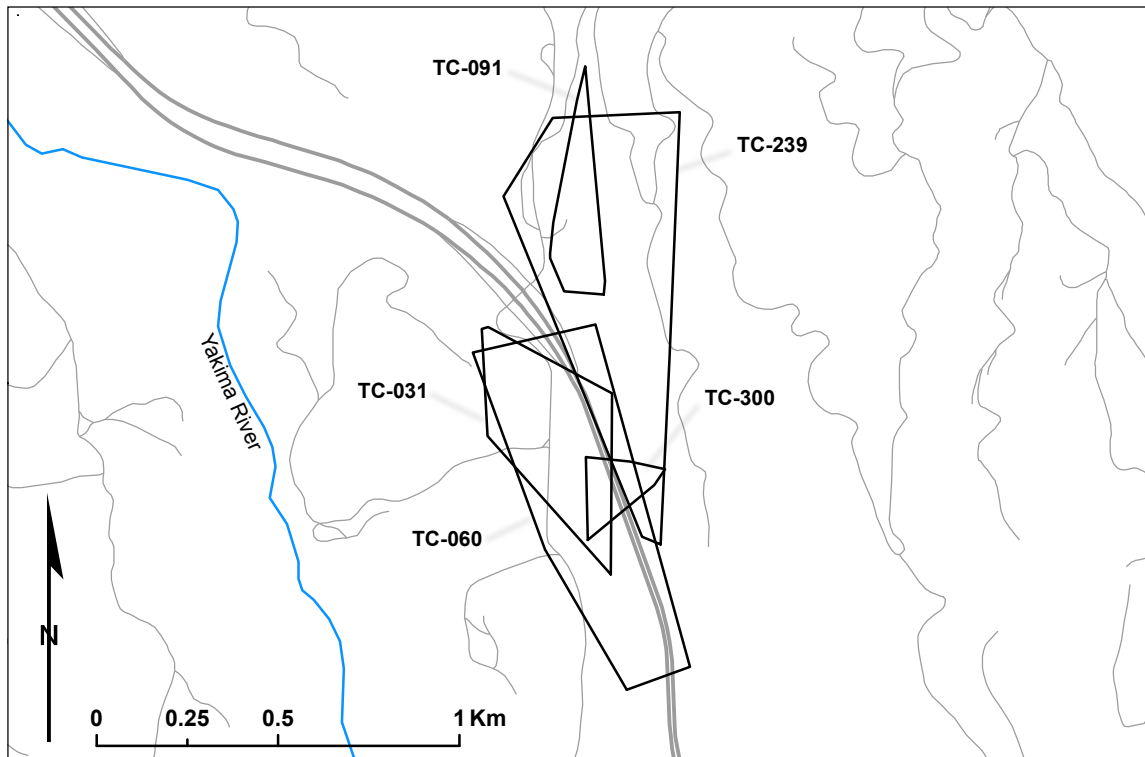


Figure 3. Simple minimum convex polygon home ranges of squirrels tracked at Toll Creek. The thick double line shows the position of the east- and west-bound lanes of I-90. Thin gray lines indicate on- and off-ramps as well as smaller paved and unpaved roads.

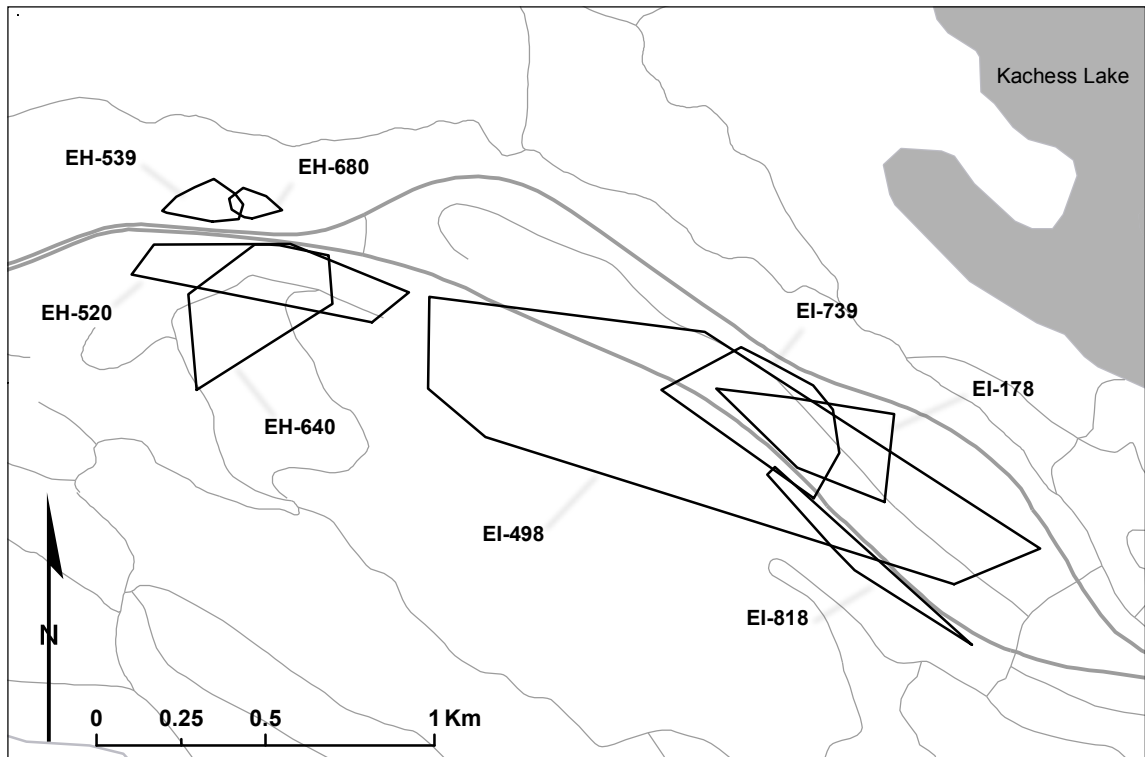


Figure 4. Simple minimum convex polygon home ranges of squirrels tracked at Easton Hill (left) and Easton Island (right). The thick double line shows the position of the east- and west-bound lanes of I-90. At this section, the east- and west-bound lanes separate for about 2.7 km and the vegetated median is > 300 m in places. Thin gray lines indicate smaller paved and unpaved roads.

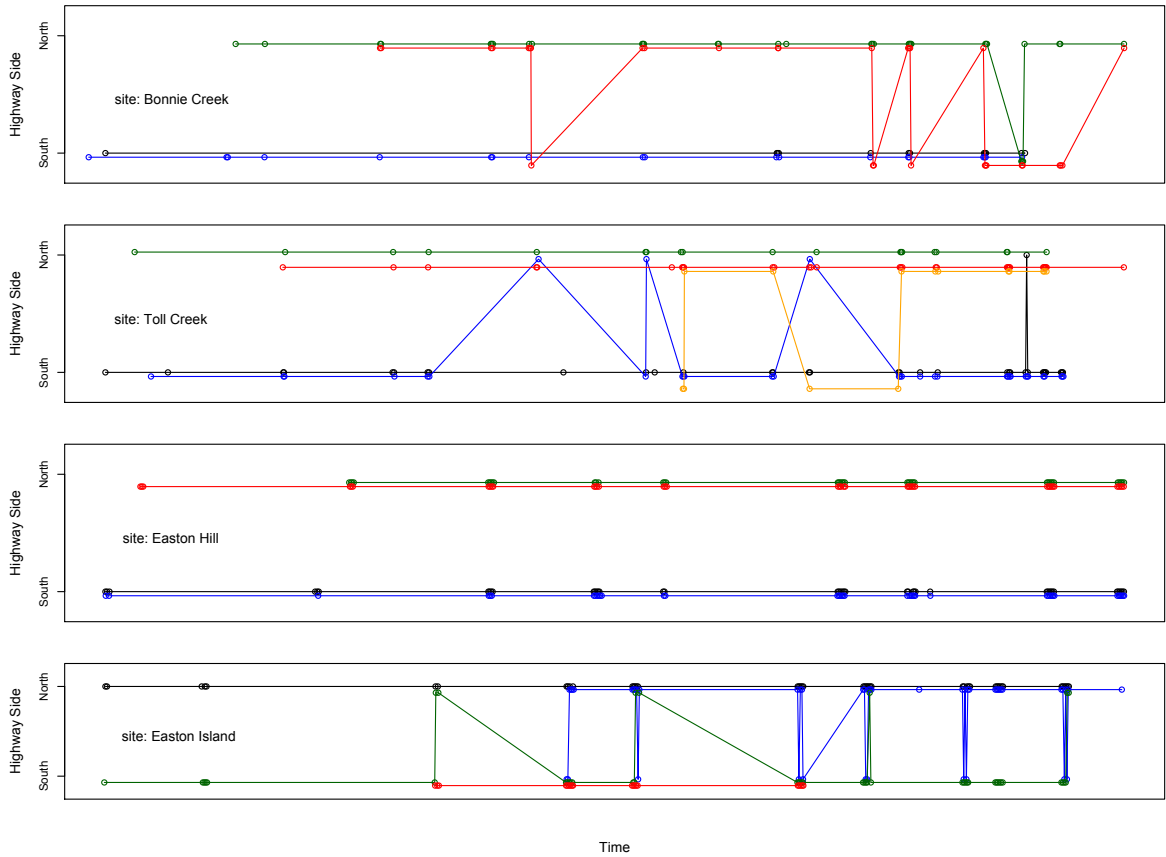


Figure 5. Locations of all radio-tracked squirrels relative to the highway over the duration of the tracking period. Each squirrel is represented by a different-colored line at each site. Sites appear, from top to bottom, in order from NW to SE along I-90.

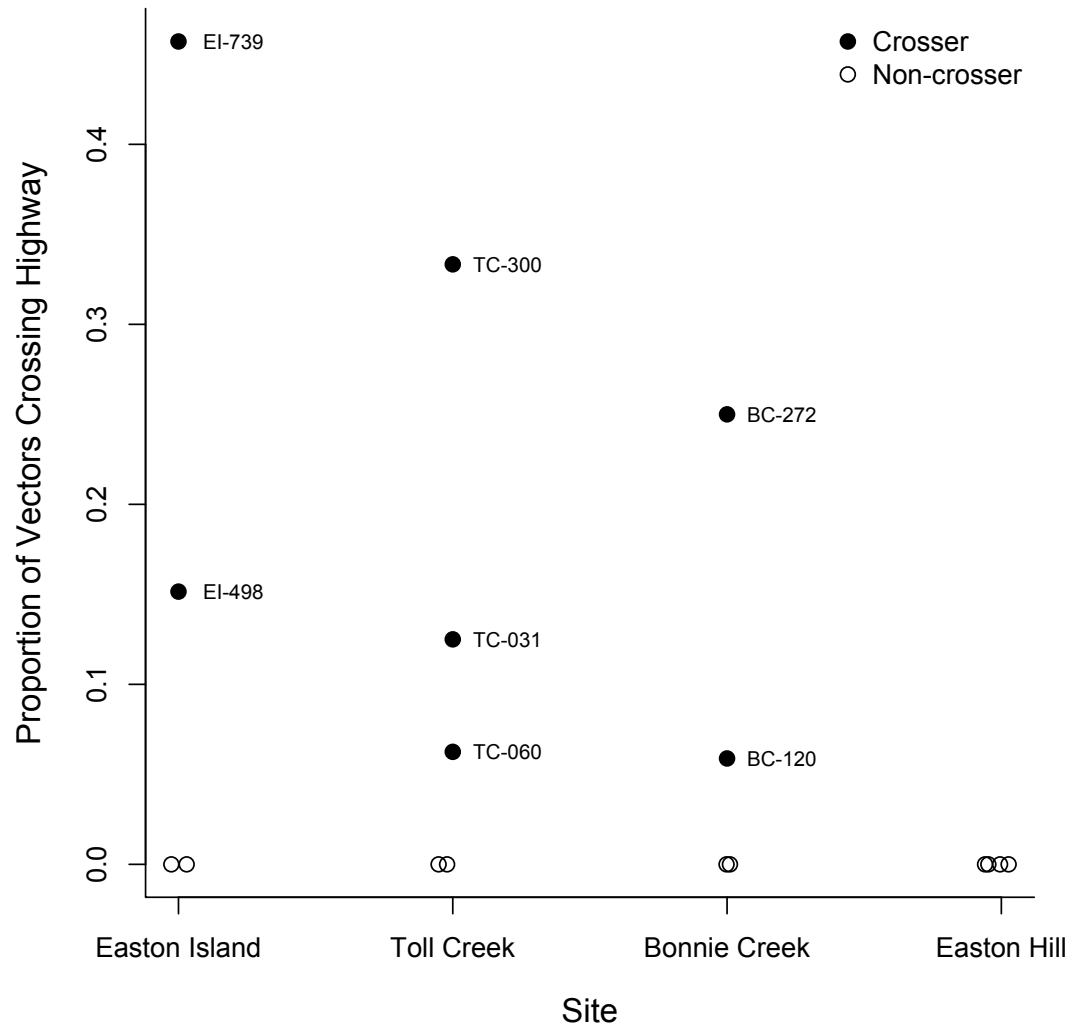


Figure 6. Crossing rates of all tracked squirrels by site. Sites are ordered from narrowest to widest average canopy gap width. Approximate average and minimum gap widths: Easton Island (57, 42); Toll Creek (72, 51); Bonnie Creek (76, 64); Easton Hill (83, 65). Gaps were measured using digital orthophotos in ArcGIS 10 (Esri, Redlands, CA, USA).

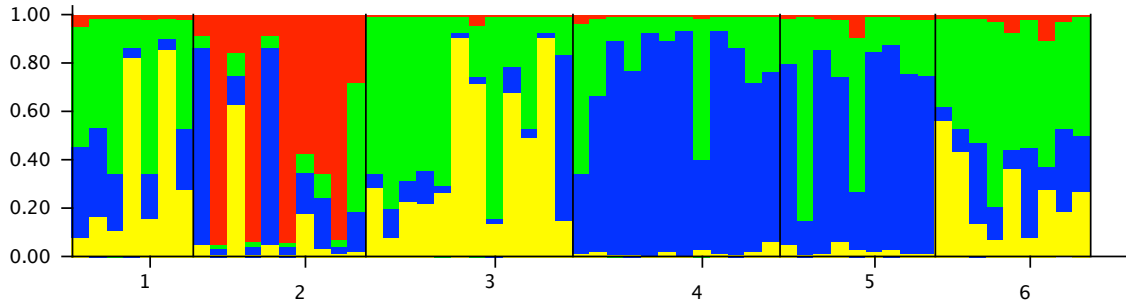


Figure 7. Results of STRUCTURE clustering analysis. Each vertical bar represents an individual, broken into K colored segments with lengths proportional to the likelihood of that individual's membership in each of the 4 populations identified by STRUCTURE. Numbers along the bottom indicate the site of capture (1 = Bonnie Cr. North; 2 = Bonnie Cr. South; 3 = Toll Cr. North; 4 = Toll Cr. South; 5 = Easton Hill North; 6 = Easton Hill South).

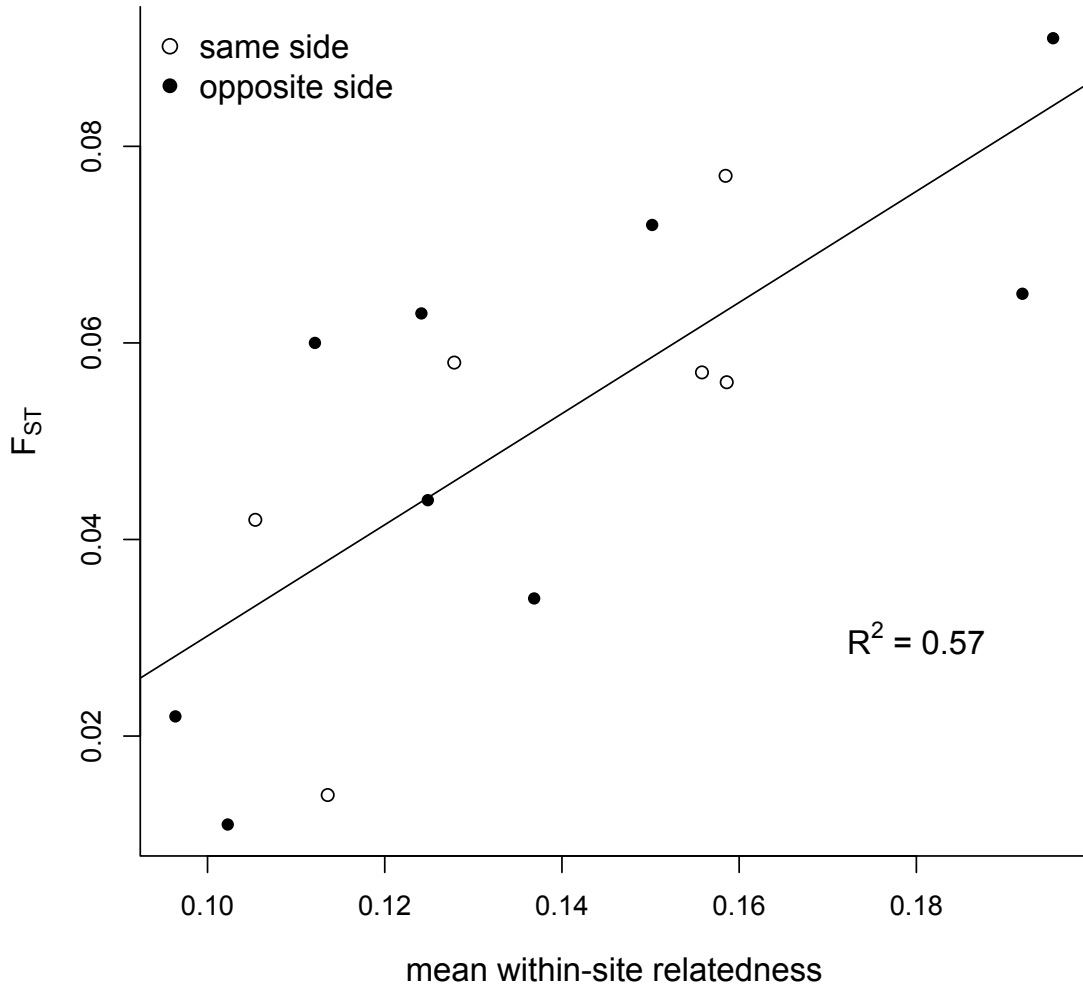


Figure 8. Mean within-site relatedness and pairwise F_{ST} for all pairs of sites. Higher within-site relatedness is significantly positively associated with estimated genetic distance between sites ($t = 4.14$ on 13 degrees of freedom, $p = 0.001$). Within-site relatedness of pairs of sites explained approximately 57% of the variation in pairwise F_{ST} . Within-site relatedness at each site is reported in Table 2.

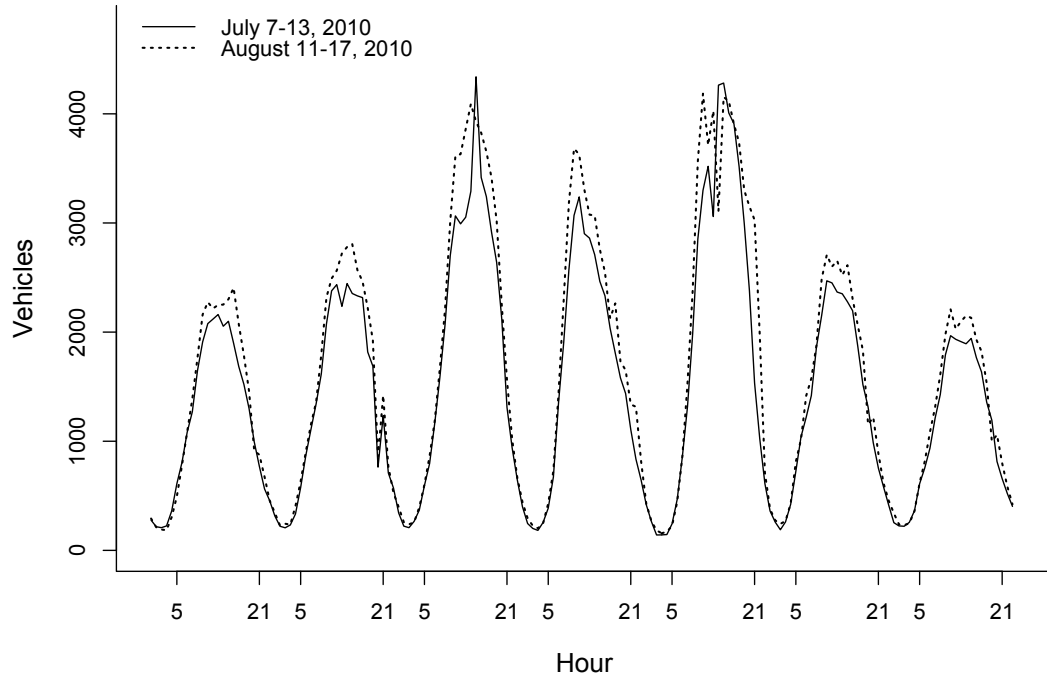


Figure 9. Daily traffic fluctuations during two randomly chosen weeks during the tracking period in 2010 at Milepost 64 (near Toll Creek sites). Squirrels were generally active during the period between about 21:00 and 05:00, indicated by tick marks on the x-axis. These periods correspond well with daily minimum traffic volumes. Data from J. Hawkins, WSDOT, personal communication, available from the WSDOT Statewide Travel & Collision Data Office.

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APPENDICES

APPENDIX A

SUMMARY OF MICROSATELLITE LOCI

Locus	Repeat motif	Primer Sequences	Size range (bp)	N _a	H _o /H _E	Accession no.
000194	(AG)15	F:TGTCCACATGAGGTTAGTACTTG R:TCTGACAAGCGCTGCCATAG	81–113	13	0.72/0.74	JF920122
001367	(AC)14	F:GATCCAGCCGCCTAGAGAAC R:TCTATGGGAGTCAATACATAAGCAG	89–105	8	0.60/0.64	JF920123
002035	(CA)13	F:TGAAAACAACCTAAGCACCTG R:TGTGACAGAGTAGCACGTTG	97–110	6	0.26/0.28	JF920124
002565	(AC)13	F:GCAGCTCAGAGTTTTGGGTG R:CCCCAGCCCACTTCTAACC	147–154	5	0.79/0.70	JF920125
004185	(GT)12	F:GAACTCTCTCTGGGCTAGTGG R:CTGTCAGGCCAACAGTTTCTC	113–124	6	0.48/0.52	JF920126
004361	(GT)13	F:TCCCTGTTTCCCCACAGATG R:GTCATGGGAAGTGCTTGACC	167–188	7	0.57/0.47	JF920127
004732	(TG)12	F:TCTATTTTGGGCACTAATTTTCAGAC R:GGAAGGATTTGTGCTTGGTATC	109–121	8	0.48/0.49	JF920128
005265	(AC)15	F:AACCAAGACAGCTATGGCAC R:CCACTGCCTCCCAAGAGTAG	232–240	7	0.76/0.69	JF920129
005430	(CA)12	F:GCACCGTATCTGCAACTCAC R:TGTCTCTAGCACGCTCTGTC	105–112	7	0.75/0.65	JF920130
006777	(GT)13	F:TGCTTACTCCCGATGTGGTC R:TAAACCTACCCTCCCAGCAC	233–242	10	0.71/0.80	JF920131
007299	(GT)16	F:TGCCCCAGGTGTACCTATTG R:ACAGTTCTGCCACGAAAAC	106–116	8	0.74/0.77	JF920132

Table 1 Characterization of 11 microsatellite loci in *Glaucomys sabrinus*. N_a: number of alleles, H_o and H_E: observed and expected heterozygosity.

APPENDIX B

MICROSATELLITE DATA

Northern flying squirrel genotypes, by capture site (BCN, BCS, TCN, TCS, EHN, EHS)

0194

1367

2035

2565

4185

4361

4732

5265

5430

6777

7299

POP

A101487B, 987987 995997 106106 148152 113115 188176 115117 232234 109110 238238 000000

A144818B, 993995 989993 106106 148152 113115 176176 115117 232234 109110 237239 112114

A144819B, 993109 993997 106110 150152 113115 176176 117117 234236 109110 237239 108112

A197570B, 993997 993993 106106 148150 115115 178176 117117 232236 110110 236236 106110

A197573B, 993997 995997 106106 152154 113115 176176 117117 234240 110110 234237 112114

A197574B, 993997 993993 106106 148150 115115 176176 117117 232236 110110 236236 106110

A197792B, 981993 993993 106106 150154 113115 176176 115115 234236 106112 237240 112114

POP

A101511B, 981981 989989 106106 147148 113115 176188 117117 236236 106112 236238 114114

A101512B, 981987 993993 106106 152152 115115 176186 117117 236240 109114 238240 000000

A159854B, 993993 993993 106106 148148 111113 186176 113117 234234 106110 237240 000000

A159855B, 981987 993993 106106 152152 115115 176186 117117 236240 109114 238241 112116

A197568B, 981981 989989 106106 147148 113115 176188 117117 236236 106112 236238 114114

A197569B, 981981 993993 106106 150152 111111 176186 117117 236240 109110 238240 112112

A197571B, 981993 989993 106106 152152 113115 176176 117117 236240 112114 238240 110116

A197572B, 981987 993993 106106 150152 115115 176186 115117 234236 109110 238242 112114

A197779B, 981101 993993 106106 150152 111111 176186 117117 236240 109110 238240 112112

A197791B, 987995 993997 106106 150152 113113 178186 111117 234234 109114 237239 112112

POP

A101486B, 993105 993993 106106 148150 113115 176190 115117 238240 110110 236241 112112

A101508B, 105107 993998 106106 150150 113122 176178 115117 234236 109110 238238 112112

A101513B, 987103 993997 106106 148150 113113 176176 117121 236236 112112 235238 114116

A101514B, 993999 993995 106106 148152 113115 176176 115115 230232 110112 237239 114116

A144820B, 987993 989991 106110 148150 115115 176178 117117 234238 110112 240241 112114

A144821B, 993993 993993 106106 148148 113113 176186 113117 234236 110112 236236 106114

A144822B, 987993 993993 106106 148150 113113 176186 117117 234240 110112 236238 106116

A144825B, 993997 991993 106110 147150 115115 167188 115117 234234 110114 238238 110112

A144827B, 993993 989995 106110 148150 113113 176176 117117 232234 110112 240240 106116

A144829B, 993105 993998 106106 148152 113115 176186 115117 232236 105112 234240 106110

A191969B, 993993 993993 106106 148150 113113 176186 117117 234236 110112 236240 106114

A191973B, 981993 989993 106106 148152 113113 176176 115117 232234 109112 238239 112114

POP

A101484B, 103109 989993 106106 152154 111115 176178 117117 234236 110110 238239 000000

A101485B, 981981 993993 106106 150154 113113 176176 107117 232234 109110 237242 112114

A173828B, 981993 989997 106108 148150 113113 176190 115117 234236 106110 236238 997112

A173829B, 105113 989995 106106 148152 113113 176176 115117 236236 106110 236239 108118

A173830B, 981981 993997 108112 148152 113113 176190 117117 234236 106109 238239 114114

A173831B, 981993 989993 106112 148150 113113 176190 117117 234234 109110 236238 997114

A173834B, 981981 995997 106108 152152 113113 176176 117117 234236 000000 239239 112114
 A173835B, 997103 993995 106106 148154 113115 176178 117117 236236 106110 238241 112118
 A173840B, 981993 989995 106106 148152 113113 176176 113117 232236 106110 239239 114114
 A173841B, 981993 993995 106110 148152 113113 176176 117119 236236 106106 239239 112114
 A197567B, 981105 989993 106112 148150 113122 176178 113117 234236 109110 236238 112112
 A197775B, 993107 993995 106106 148148 113113 176178 117117 234234 109110 238238 000000
 POP
 A144823B, 993105 995997 106106 150152 113115 176176 117117 232234 000000 236242 110114
 A144824B, 109113 102102 110110 152152 000000 176182 113113 222222 106106 233235 000000
 A173832B, 981993 995997 106106 150152 113113 176188 115117 232234 109110 238238 112114
 A173833B, 981993 989993 106106 148150 113113 176176 117117 232234 110110 238241 112116
 A173839B, 981101 102105 106106 150150 113115 176176 115117 236236 110110 238240 112116
 A173886B, 993105 995997 106106 150152 113113 176176 115117 232234 106109 236242 110114
 A173887B, 981105 989995 106106 148150 113113 176176 117117 232236 106109 236236 108110
 A173894B, 981981 993995 106106 150152 113122 176182 115117 232236 106110 238238 112116
 A173901B, 981981 993995 106106 150152 113122 176182 115117 232236 106110 238238 112116
 POP
 A166789B, 985993 993993 997106 148148 113120 176176 113117 236236 110110 238240 106110
 A166790B, 993993 993993 106110 148152 120122 176178 113115 234236 106110 236240 106112
 A173558B, 981993 989993 106110 152152 113120 176178 113117 234236 106110 236239 112112
 A173881B, 987993 991993 108114 150152 113115 176188 115117 234236 109110 238238 108112
 A173884B, 995101 993993 106106 147150 113115 176186 117117 232234 110110 236240 112112
 A173885B, 981981 989989 106110 150152 113113 176182 117117 234236 110116 236241 116116
 A173899B, 997997 993993 106106 150150 115115 176176 115117 236236 109110 238238 110112
 A173902B, 981993 993993 106110 148152 113113 176176 115115 234236 109110 236238 112112
 A173904B, 993993 989993 106106 147152 113124 176176 109117 232236 110110 236239 110114