Impact of Landscape Management on the Genetic Structure of Red Squirrel Populations

Marie L. Hale,[1]* Peter W. W. Lurz,[2] Mark D. F. Shirley,[2] Steven Rushton,[2] Robin M. Fuller,[3] Kirsten Wolff[1]

Landscape management practices that alter the degree of habitat fragmentation can significantly affect the genetic structure of animal populations. British red squirrels use "stepping stone" patches of habitat to move considerable distances through a fragmented habitat. Over the past few decades, the planting of a large conifer forest has connected groups of forest fragments in the north of England with those in southern Scotland. This "defragmentation" of the landscape has resulted in substantial genetic mixing of Scottish and Cumbrian genes in squirrel populations up to 100 kilometers from the site of the new forest. These results have implications for the conservation management of animal and plant species in fragmented landscapes such as those found in Britain.

Fragmentation of populations may result in loss of genetic variation, increasing the risk of population extinction (*1*, *2*). However, a mosaic of small fragments of suitable habitat may act as a single large habitat if the fragments are linked by linear corridors or "stepping stone" patches of habitat (*3*, *4*). Despite a dramatic decline in populations elsewhere (*5*), red squirrels (*Sciurus vulgaris*) are still relatively abundant in the north of England and the Scottish Border region and occupy a patchwork of highly fragmented woodland blocks (*6*). Using maps of woodland fragments (*7*), we analyzed the genetic composition of red squirrel populations defined by interpatch distance, to determine whether squirrels are able to use a mosaic of forest fragments as a single habitat. Additionally, over the past few decades, the planting of a large new forest (Kielder Forest) has connected groups of forest fragments in northern England with those in southern Scotland (*8*). Using museum specimens collected over the past century, we examined the impact of this habitat defragmentation on the genetic composition of British red squirrel populations.

All dried *S. vulgaris* skin specimens at both the Hancock Museum and Tullie House were sampled, resulting in a total of 102 individuals. Four polymorphic microsatellite loci (Scv3, Scv8, Scv9, and Scv10, with three to nine alleles per locus) were amplified for each individual (*9*) [Web table 1 (*10*)], and the resulting multilocus genotypes were used to determine both geographic and temporal genetic structure.

The extreme fragmentation of its habitat makes it difficult to determine what constitutes a squirrel population. Squirrels are capable of moving and dispersing between patches of woodland but avoid crossing open areas during dispersal movements if possible (*11*, *12*). Woods that were within a certain "linking" distance were considered to represent a single habitat occupied by a single population, and individuals were assigned to populations on the basis of which wood they were collected in. Four linking distances were analyzed: 3.0, 2.5, 2.0, and 1.5 km (*13*, *14*). These distances were chosen because at a linking distance greater than 3.0 km, all woodlots formed one large group, and distances below 1.5 km resulted in extremely small samples. To maximize the use of the limited samples, all populations containing at least two individuals were used in the analyses [Web table 2 (*10*)].

Comparison of genetic subdivision of populations at different woodlot linking distances shows how far squirrels can move through unfavorable habitat. F_{ST} (the proportion of total genetic variation among populations, assuming the infinite allele model), calculated with ARLEQUIN (*15*), was used as a measure of the degree of population subdivision because it is less biased and is a more conservative estimator of gene flow than R_{ST} (the proportion of total genetic variation among populations, assuming the stepwise mutation model), when sample numbers and the number of loci are small (*16*). Significant genetic subdivision exists among groups at all four woodlot linking distances (3.0 km: $F_{ST} = 0.063$, $P = 0.027$; 2.5 km: $F_{ST} = 0.141$, $P < 0.0001$; 2.0 km: $F_{ST} = 0.146$, $P < 0.0001$; and 1.5 km: $F_{ST} = 0.161$, $P < 0.0001$). The F_{ST} value increases as woodlot linking distance decreases, suggesting that the maximum dispersal distance of this species over unfavorable ground is probably less than or equal to 1.5 km. At greater linking distances, the within-population variation was elevated and the F_{ST} value decreased artificially because of the pooling of heterogeneous samples. All further analyses were on populations defined with a linking distance of 1.5 km, because the F_{ST} values suggest that this distance more accurately defines *S. vulgaris* populations.

The specimens were collected between 1918 and 2000, with most samples collected since 1960 [Web table 3 (*10*)]. Temporal variation could only be analyzed within the Cumbria and Tyne Valley populations, because these two samples were large enough ($n = 31$ and 30, respectively) to allow further subdivision. Individuals were pooled into decades based on specimen collection date, and genetic variation was compared between decades. There was no change in the genetic composition of the Tyne Valley sample over the time period from 1960 to 2000 ($F_{ST} = -0.015$, $P = 0.728$); however, the genetic composition of the Cumbrian sample changed significantly over a similar time period ($F_{ST} = 0.148$, $P < 0.0001$). All Cumbrian samples collected after the 1980s were significantly different from all Cumbrian samples collected before the 1980s. When samples were pooled into two groups (pre-1980, $n = 9$; and post-1980, $n = 21$), F_{ST} increased to 0.170 ($P < 0.0001$), supporting the premise of a substantial change in gene frequencies during the 1980s.

Both pairwise F_{ST} and Nei's standard genetic distance (D_S) were used as measures of genetic distance between populations [Web table 4 (*10*)]. The pattern of subdivision in both pairwise F_{ST} and D_S suggested three genetic regions: northern, western, and eastern, with the grouping of Cumbria altering from a western population to a northern population during the 1980s (Fig. 1). The distribution of genetic variation was therefore recalculated with three levels: within populations, between populations within regions, and between regions. The largest portion of the total genetic subdivision ($F_{ST} = 0.215$, $P < 0.0001$) was found between the three regions [$F_{CT} = 0.165$ (C, region; T, total), $P < 0.0001$], with much less subdivision between populations within regions [$F_{SC} = 0.059$ (S, populations), $P < 0.0001$]. The reality of the three genetic regions was further tested statistically with the genotype as-

[1]Department of Agricultural and Environmental Science, [2]Centre for Life Science Modelling, University of Newcastle, Newcastle-upon-Tyne, NE1 7RU, UK. [3]Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, Huntingdon, PE28 2LS, UK.

*To whom correspondence should be addressed. E-mail: m.l.hale@ncl.ac.uk

signment test (*17–19*). This test makes use of multilocus information. It determines the probability of each individual genotype occurring in each population and assigns the genotype to the population with the highest probability. The significance of the number of correct assignments is tested with a permutation procedure, with the null hypothesis that the groups were sampled from a single well-mixed population (*20*). Most individuals were correctly assigned to each of the three regions (northern: 24 out of 37 individuals, $P = 0.001$; eastern: 34 out of 42, $P < 0.001$; western: 12 out of 13, $P < 0.001$) (Fig. 2). Genotype assignment was also used to assess the reality of the apparent switch in gene frequencies in Cumbria during the 1980s. Pre-1980 Cumbrian squirrels were assigned to the western group in 89% of cases (eight out of nine), whereas post-1980 Cumbrian squirrels were mostly assigned to the northern group (76%, or 16 out of 21 individuals) (Fig. 2).

This startling change in genetic composition has occurred in conjunction with a change in woodlot coverage in this area (Fig. 3). Kielder Forest was initially planted in the 1920s, but it was not until the 1950s and 1960s that expansion of this forest created a link between the western and northern forest fragments (*8*). The 20- to 30-year lag between the linking of forest fragments and the change in squirrel genetic composition is probably due to the time it takes the major tree species planted (Sitka and Norway spruce) to mature (*21*) and provide a suitable squirrel habitat.

The apparent one-way migration from north to west is, perhaps, not surprising given the size of Kielder Forest (50,000 ha) (*8*), as compared with the much smaller combined area of forest inhabited by the Cumbrian population (approximately 8800 ha). Although population density estimates are slightly lower in Kielder than Cumbria (0.02 to 0.42 and 0.13 to 1.3 squirrels per hectare, respectively) (*22*, *23*), the direction of migration is expected to be biased because of the much larger squirrel population in Kielder Forest as compared with the combined populations in forest fragments in Cumbria.

The rapid movement of northern genes through the western population suggests that the original population of *S. vulgaris*, before human-made fragmentation, would probably have been a single relatively panmictic population. Therefore, the integration of the Cumbrian population into the northern group is not only increasing genetic diversity within the Cumbrian population (mean heterozygosity increased from 0.333 to 0.488) [Web table 1 (*10*)], but is also returning red squirrel populations in this region to their "original" state in terms of genetic structure. However, although increased connectivity may increase genetic diversity in squirrel populations, it also increases the risk of disease transmission. This may be a problem in British red squirrel populations, in which a parapox virus has been known to cause local population extinction (*24*).

Generally, gene flow and the degree of population differentiation have been estimated from a single sampling episode (*25*) or through modeling of dispersal behavior (*26*).

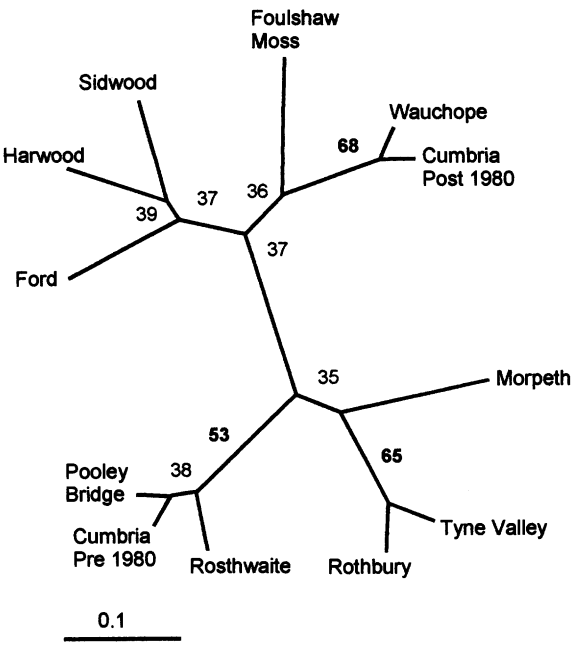

Fig. 1. Bootstrapped UPGMA tree of D_S (*27*), constructed with the GENDIST, NEIGHBOR, and CONSENSE programs in PHYLIP version 3.57c (*28*) and visualized with TREEVIEW (*29*). Numbers specify bootstrap values (100 replications); bootstrap values over 50 are in bold. Bootstrap values are fairly low; however, the three genetic groups are evident and are supported statistically by the genotype assignment test. Morpeth, Rothbury, and Tyne Valley represent populations on the east coast; Cumbria, Pooley Bridge, and Rosthwaite represent the west coast; and Ford, Harwood, Sidwood, and Wauchope (all of which are in the north of England) and Foulshaw Moss in the southern Lake District (southwest) represent the northern cluster. The inclusion of Foulshaw Moss in the northern group is probably the result of the well-documented translocation of *S. vulgaris* individuals from Europe into both Scotland and southern Cumbria/Lancashire (*30*).

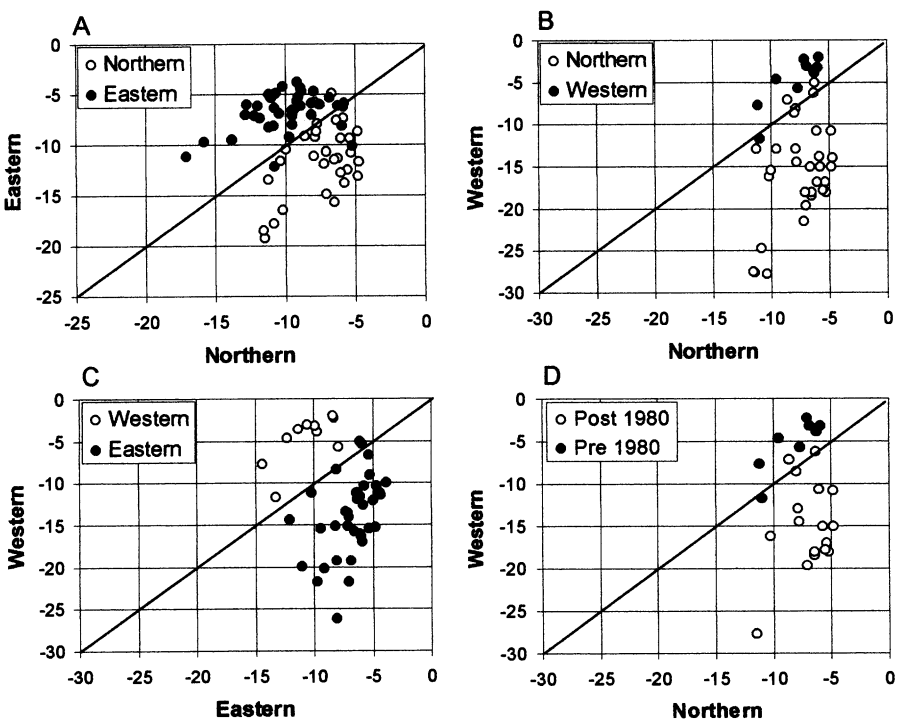

Fig. 2. Scatter plots of log likelihoods of population membership (*18*, *19*) for each possible pair of genetic groups. (**A**) Northern versus eastern genetic group. (**B**) Northern versus western. (**C**) Eastern versus western. (**D**) Assignment of Cumbrian squirrels to the northern and western groups. Circles represent individual genotypes, coded on the basis of the region they were collected in. Plots are split into two halves representing two regions, and genotypes are correctly assigned where they fall on the same side of the 45° line as the region they were collected in. Most genotypes were assigned correctly, and the separation between pre-1980 and post-1980 Cumbrian samples is clear, with most pre-1980 specimens assigned to the western group and post-1980 specimens to the northern group.

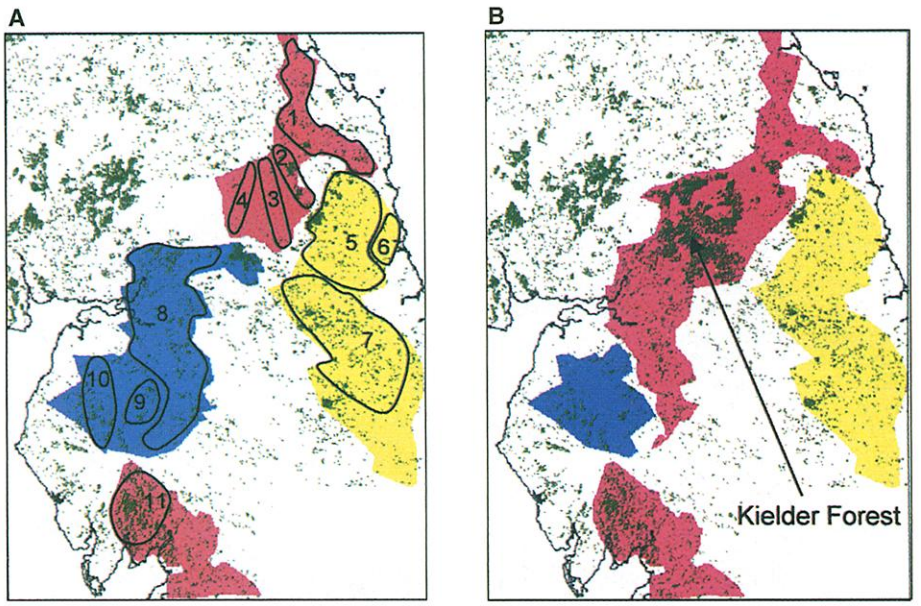

Fig. 3. Change in genetic composition and woodland coverage. (**A**) Woodland coverage in the absence of Kielder Forest is represented in dark green, with the three basic red squirrel genetic groups color coded. Red = northern group: Ford (1) (n = 4 individuals), Harwood (2) (n = 2), Sidwood (3) (n = 2), and Wauchope (4) (n = 2), plus Foulshaw Moss (11) (n = 5). Yellow = eastern group: Rothbury (5) (n = 10), Morpeth (6) (n = 2), and Tyne Valley (7) (n = 30). Blue = western group: Cumbria (8) (n = 31), Pooley Bridge (9) (n = 2), and Rosthwaite (10) (n = 2). The colored areas include all woods within the linking distance of 1.5 km to woods where squirrels were sampled. Black outlines indicate the area over which specimens in each population were collected. (**B**) Woodland coverage including Kielder Forest. Again, the three main groups are color coded as above. Cumbria (8) now forms part of the northern genetic group.

Our study takes advantage of large changes in habitat fragmentation and accurate maps and samples over the same period, enabling us to show the importance of habitat patches in wild populations as avenues for dispersal. The northern genes have leapfrogged through hundreds of forest fragments in a period of 20 years, demonstrating the use of stepping stone patches of forest by red squirrels. These findings suggest that where a network of stepping stones is available within a critical dispersal distance, gene flow can be very rapid through highly fragmented landscapes. It also indicates that human-made changes affecting the connectivity of a landscape can result in changes in genetic structure, not only in the area of habitat change but in populations hundreds of kilometers from the site of habitat change.

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Effects of Size and Temperature on Metabolic Rate

James F. Gillooly,[1]* James H. Brown,[1,2] Geoffrey B. West,[2,3] Van M. Savage,[2,3] Eric L. Charnov[1]

We derive a general model, based on principles of biochemical kinetics and allometry, that characterizes the effects of temperature and body mass on metabolic rate. The model fits metabolic rates of microbes, ectotherms, endotherms (including those in hibernation), and plants in temperatures ranging from 0° to 40°C. Mass- and temperature-compensated resting metabolic rates of all organisms are similar: The lowest (for unicellular organisms and plants) is separated from the highest (for endothermic vertebrates) by a factor of about 20. Temperature and body size are primary determinants of biological time and ecological roles.

Metabolism sustains life. It is the process by which energy and materials are transformed within an organism and exchanged between the organism and its environment. Whole organism metabolic rate scales with the 3/4-power of body mass and increases exponentially with temperature (*1*, *2*). The effect of temperature on a biological process is tradi-