

## Disease threats posed by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain

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### SUMMARY

Red squirrels are declining in the United Kingdom. Competition from, and squirrel poxvirus (SQPV) disease carried by, grey squirrels are assumed to be determining the decline. We analyse the incidence of disease and changes in distribution of the two species in Cumbria, from 1993 to 2003 and compare these to the predictions of an individual-based (IB) spatially explicit disease model simulating the dynamics of both squirrel species and SQPV in the landscape. Grey squirrels increased whilst red squirrels declined over 10 years. The incidence of disease in red squirrels was related to the time since grey squirrels arrived in the landscape. Analysis of rates of decline in red squirrel populations in other areas showed that declines are 17–25 times higher in regions where SQPV is present in grey squirrel populations than in those where it is not. The IB model predicted spatial overlap of 3–4 years between the species that was also observed in the field. The model predictions matched the observed data best when contact rates and rates of infection between the two species were low. The model predicted that a grey squirrel population control of >60% effective kill was needed to stop the decline in red squirrel populations in Cumbria.

### INTRODUCTION

The red squirrel (*Sciurus vulgaris* L.) is considered an endangered species in the Britain. Its decline has been associated with the spread of the grey squirrel (*S. carolinensis* Gmelin) an alien species introduced from North America between 1976 and 1929 [1, 2]. The grey squirrel is still spreading and red squirrel populations invariably decline after grey squirrels

colonize an area. Both squirrel species utilize similar food resources (tree seeds) and woodland habitats and inter-specific competition has been demonstrated as a plausible mechanism for the decline [3–5]. In the 1980s an emerging infectious disease (EID [6]) was identified in red squirrels, which may also play an important role in their decline. The disease is caused by a poxvirus [7, 8]. This virus was initially thought to be a member of the genus *Parapoxvirus* but which was distinct from parapoxviruses found in seals, sheep and cattle [9]. More recent studies, however, indicate that the poxvirus represents a previously unrecognized genus of the Chordopoxviridae [10],

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and here we shall refer to it simply as squirrel poxvirus (SQPV). When red squirrels become infected by SQPV, disease ensues and the probability of death within  $\sim 2$  weeks is very high [11–14]. In contrast SQPV appears benign in grey squirrels; they show an antibody response but do not develop signs of disease [14]. Grey squirrel populations in England and Wales show seroprevalence rates ranging from 42 to 100%, but those in Scotland and parts of northern England do not appear to have been infected [13]. It has been concluded [13] that grey squirrels act as reservoir hosts for the virus and it has been demonstrated that grey squirrels are able to cause disease in red squirrels in captivity [14]. Grey squirrels as reservoir hosts would explain the observed, transient disease epidemics with high mortality rates in small and fragmented red squirrel populations, and provide a better explanation for the rapid decline in the red squirrels in England.

The origin of SQPV is unclear; it is likely to have been introduced into Britain from North America with the grey squirrel, but it is possible that grey squirrels have become a reservoir host to an existing pathogen subsequent to their arrival (see refs [1, 2]). Importantly, Sainsbury & Gurnell [11] noted that recent outbreaks of SQPV disease in red squirrels seemed to occur after the arrival of grey squirrels in the vicinity. In this paper we seek to establish whether there is a link between the spread of grey squirrels, SQPV and the decline of the red squirrel, and if so, whether the rate of decline of red squirrels and the rate of spread of grey squirrels is increased by the presence of SQPV originating in the grey squirrels. We consider the current spread of grey squirrels in Cumbria, England and quantify the association of this spread with documented outbreaks of SQPV disease in red squirrel populations. Since direct experimentation on virus transmission between the two species is technically difficult and ethically questionable (the red squirrel is a protected species in Britain), we use a combined field survey and population modelling approach to characterize the demography of the two species and to examine the role of disease in the replacement process. In particular, we: (i) examine whether the observed presence of grey squirrels is responsible for outbreaks of SQPV disease in red squirrels in Cumbria; (ii) compare the rates of decline in Cumbria to regions where grey and red squirrels have been in contact but SQPV has not been present in grey squirrels; (iii) develop an individual-based (IB) model of squirrel and disease dynamics

to investigate the extent to which disease could be responsible for the decline of red squirrels in Cumbria. Last, we consider the implications of our findings for policy makers trying to manage the disease threat by investigating the distribution and level of grey squirrel control needed to stop the decline of red squirrels in Cumbria.

## METHODS

### Data collection

Records of red and grey squirrel distribution in Cumbria over the period 1993 to 2003 were collected from a range of sources. The key sources were local records held at the Tullie House Museum in Carlisle and from an appeal for records of sightings from the public instigated from 1993 by Red Alert North West (RANW), a red squirrel conservation organization. Records also included dead squirrels, either from trapping, road kills, or any other causes of mortality.

### Poxvirus infection and disease data

Data on SQPV disease cases in red squirrels were based on post-mortem examination results. Disease was identified using clinical signs [12] and confirmed by electron microscopy (EM) of skin lesions where this was possible [7]. Data on infection in grey squirrels was provided by testing blood samples for SQPV antibodies taken from squirrels in locations where trapping was possible. Serum samples taken from corpses of both species were tested for antibody to SQPV using an ELISA [13].

### Generating maps of the distribution of red and grey squirrels in Cumbria

For each squirrel species records of presence for each year were collated and entered into a geographic information system, GIS (GRASS [15]) at 100 m resolution. Annual distribution maps at 2000 m resolution (tetrads) within the UK National Grid were created. The collection of records was not based on systematic sampling, therefore records were not available for each tetrad for every year. We used the methodology of Reynolds [16] to create maps. In this we assumed that:

- (i) red squirrels that were recorded in any one tetrad for at least three over the course of 1993 to 2003 were considered to have been present in that

tetrad for all years from 1993 to the last recorded year, after which they were assumed to become extinct;

- (ii) grey squirrels were considered to have been present in a tetrad in all years subsequent to the first year in which they were found present.

The affects of these assumptions on the results are considered below.

Maps of where both species coexisted in Cumbria were created by overlaying the maps for each species each year. Records of the disease in the red squirrel population and the presence of antibodies to squirrel poxvirus in grey squirrels were then used to create equivalent annual maps of the spatial distribution of the virus in the two species. We used the occurrence of poxvirus as a response variable in logistic regression to investigate whether duration of coexistence, the presence of seropositive grey squirrels and the area of available habitat were significant predictors of disease incidence in red squirrels.

#### **Rates of decline in red squirrels in areas with and without SQPV**

We calculated the average annual decline (km<sup>2</sup>) of red squirrels sympatric with grey squirrels from observed published records for areas reported to have no disease (Italy and Scotland) and contrasted these with areas that are known to have had SQPV outbreaks (Norfolk and Cumbria).

#### **Population dynamics model**

We used a spatially explicit, IB model to investigate the population dynamics of red and grey squirrels and poxvirus disease in populations of both species of squirrel in Cumbria. A published GIS population model [17, 18] originally applied in a *post hoc* analysis of red squirrel decline, was used to simulate the spread of poxvirus and mortality in both species. Briefly, the model had two main components. First, the GIS stored environmental, habitat and population information for squirrels of each species in the landscape. Second, an IB population dynamics module simulated individual life histories and dispersal within the GIS-held landscape. The population model was written in the programming language C and integrated with the GIS component through a UNIX-shell environment. The model was stage-structured [19], in so far as discrete stages were recognized in the population, but the life-history processes of mortality, fecundity and

dispersal were modelled stochastically at the level of the individual within the different age classes in each population. The outputs of the model were the population of individuals of each species and their disease status in each individual habitat block in the landscape.

#### **Defining habitat suitability, use and carrying capacity of habitat blocks in the GIS**

The land surface was partitioned into two: (a) areas of habitat that could be used by the two squirrel species as home-ranges for residing, foraging and breeding; and (b) areas through which animals could move when dispersing, but which they could not exploit for home ranges. Identification of habitat suitable for occupation by squirrels within the landscape was undertaken using the GIS. Suitable habitats were defined as scrub, deciduous and coniferous woodland as identified from the categories of the ITE Land Cover Map (derived from a classification of Landsat satellite imagery [20]). Available habitat in the landscape was predicted in terms of cells (pixels) of 25 × 25 m. All contiguous habitat cells were then aggregated to form habitat blocks.

#### **Population dynamics and dispersal of red and grey squirrels in landscapes**

Each block of suitable habitat within the landscape had separate populations of red and grey squirrels. Two age classes were modelled, adults and juveniles. Populations in each woodland block had their own dynamics that interacted through the processes of dispersal. For each block of habitat, in each year, population size was modelled in terms of net change resulting from four processes: gains from breeding recruitment and immigration from other habitat parcels, and losses due to adult and juvenile mortality and emigration. Reproduction was assumed to occur in two waves, in spring and summer [3, 4]. Litter size was varied as a truncated Poisson deviate with the mean varied as an input. Mortality was assumed to occur after all breeding had finished and was modelled at the level of the individual as a stochastic process [18]. We assumed that population size in each block was determined by a carrying capacity for each species estimated from known space requirements [18]. If the population exceeded the carrying capacity, animals were forced to disperse to other habitat blocks, or die if there was no suitable available

Table 1. (a) Life history and (b) infection parameter ranges used in the sensitivity analysis of the squirrel poxvirus population dynamics model for red and grey squirrels

	Minimum	Maximum	Reference
<i>(a) Squirrel life history parameter</i>			
Dispersal distance (m)	10 000	20 000	[21]
Fecundity	2	6	[22–25]
Juvenile mortality	0.4	0.8	[3, 21, 26]
Adult mortality	0.2	0.6	[3, 21, 26]
Proportion females with 1st litter	0.5	1.0	[22, 27–29]
Proportion females with 2nd litter	0.15	0.5	[22, 27–29]
<i>(b) Infection parameters</i>			
Encounter rate	0.01	0.2	No published data
Infection probability	0.01	1.0	No published data

habitat. Dispersal of individuals was modelled as a process occurring once a year in autumn and was assumed to occur as a result of intra-specific competition [4]. Animals in each block were either infected with poxvirus or non-infected. All animals that were infected were assumed to be infectious.

#### Modelling disease spread in relation to population density and transmission probability

For each habitat block in the landscape, the spread of poxvirus among individuals in the population over the course of a year was simulated as follows:

- (i) The likelihood that each healthy squirrel (red or grey) would encounter an infected squirrel in the habitat block during the course of a 2-week infection period was estimated. This depended on the population densities of healthy and infected red and grey squirrels present at the beginning of the time period and was calculated as the product of the encounter rate and the proportion of infected squirrels in the combined total population of both species. We modelled encounter rates to ensure that the chance of an animal being infected in a population would increase with the population density of infected animals present in the habitat block.
- (ii) The likelihood that animals that had been exposed during an encounter would become infected was determined as the product of the encounter probability and the probability of infection. This last was varied as an input.
- (iii) The likelihood that individual squirrels died following infection was determined from the disease mortality rate for the respective species.

- (iv) The model then iterated for all squirrels for each infective period, with the output of one infective time period providing the inputs for the next.

Exposure, infection and mortality processes were modelled stochastically for each individual. The likelihood of exposure, infection and mortality for each individual were determined by sampling deviates from a uniform distribution in the range 0–1, with the process occurring if the deviate was in the modelled range of the relevant life stage. It was assumed that the only form of interaction between the two species was virus transmission.

#### Running the population and disease model

Population models based on six life-history parameters alone have been used successfully to simulate observed dynamics of squirrel populations in three areas of the United Kingdom and in Northern Italy [17, 18, 30, 31]. We used the same approach and modelled: maximum dispersal distance, adult mortality, juvenile mortality, fecundity (litter size), and the proportion of females having a first litter, and the proportion of females having a second litter in each year. We did not model inter-specific competition since previous research [17] showed that inclusion of this process did not impact on the rate of red squirrel population decline. The implications of this are considered in the Discussion section. Maxima and minima of each life-history parameter were derived from the literature (Table 1) and used as bounds for uniform distributions for each parameter. We used Latin hypercube sampling (LHS) to generate suites of life-history inputs from each distribution [17]. Five hundred sets of inputs were generated and these were

Table 2. Grey squirrel life-history parameter sets for the four best models simulating grey squirrel spread in Cumbria and their mean deviation (MD) in tetrads/year (the mean of the four best runs for each parameter was used as a model input for investigating the effects of disease on red squirrel persistence)

Parameter	Model 1	Model 2	Model 3	Model 4	Mean
Dispersal distance	11 029	10 553	11 943	11 175	11 175
Fecundity	5.0	5.0	5.0	4.0	4.75
Juvenile mortality	0.53	0.53	0.63	0.65	0.57
Adult mortality	0.34	0.23	0.31	0.36	0.31
Proportion females with 1st litter	0.79	0.97	0.81	0.90	0.91
Proportion females with 2nd litter	0.37	0.33	0.49	0.47	0.42
MD	42.3	47.8	49.2	49.0	

used to investigate the spread of the grey squirrel into Cumbria using the known distributions of the species in 1992 as starting conditions. The model used habitat data spatially referenced at a 25-m scale, whilst the field survey information was held at tetrad (2000-m) scale. Accordingly the outputs from the model were scaled up to present presence (and absence) of squirrels in Cumbria at the 2000-m scale to allow comparison between model predictions and the observed changes in the distribution of red and grey squirrels. The model was run 10 (replicate) times for each suite of inputs for a 10-year period. The mean number of tetrads occupied each year was then calculated from the 10 replicate runs and compared with observed change in the number of tetrads occupied over the period 1992–2002. The goodness of fit of predicted to observed numbers of tetrads occupied was then measured by calculating the mean deviation (MD) of numbers of predicted tetrads occupied from those observed for each suite of inputs per year as follows:

$$\text{mean deviation (MD)} = \sum_{i=1}^n \frac{|p_i - o_i|}{n},$$

where  $|p_i - o_i|$  is the modulus of the difference between the predicted and observed number of tetrads occupied by the species; and  $n$  is the total number of years. Low values represent a better fit of the model to observed data than high values. The suite of life-history parameters that lead to the closest match between predicted and observed spread of grey squirrels in Cumbria was then used to investigate the impact of disease on the distribution of red squirrels in Cumbria (Table 2). To parameterize the red squirrel model we used the life-history parameters shown in Table 3. These represent values typically encountered in

Table 3. Red squirrel life-history variables used as model input to investigate impacts of grey squirrel spread and squirrel poxvirus disease on red squirrel population decline

Parameter	Model	Observed	Reference
Fecundity	3.5	Mean 3.6 (range 1–6)	[32]
Juvenile mortality	0.6	0.60–0.85 0.75–0.85	[27, 32]
Adult mortality	0.23	0.23	[33]
Proportion females with 1st litter	0.9	0.4–1.0	[27]
Proportion females with 2nd litter	0.3	0.2–0.6	[27, 28]

‘average’ and ‘good’ years for red squirrels in the United Kingdom [3, 4]. We did not model the population dynamics under conditions typical of ‘poor’ years in order to be conservative in our assessment of the likely impact that disease would have on red squirrels. Since we had no prior information on contact rates and infection probabilities we used LHS to create 100 sets of inputs covering a range of contact rates and infection probabilities. Runs were undertaken assuming that 50% of the initial population of grey squirrels were infected, as ~50% of all grey squirrels tested from the Cumbrian population were positive for antibodies to squirrel poxvirus [13]. The model was then run 10 years for each of the disease inputs and the following variables derived from the maps created for each year in each model run:

- (i) the number of tetrads predicted to be occupied by red squirrels in each year of each run;
- (ii) the MD of the predicted number of tetrads occupied by red squirrels from the number observed in the field for each run;

Table 4. Results of a logistic regression relating the observed occurrence of diseased red squirrel records in tetrads in Cumbria to the available habitat and the duration of overlap with grey squirrel ( $n=166$ )

Predictor	Coefficient	S.D.	Z	P
Constant	-8.107	1.706	-4.75	<0.001
Area of deciduous wood	0.001048	0.000476	2.20	0.028
Years overlap with greys	0.6095	0.2085	2.92	0.003
Presence of squirrel poxvirus seropositive greys	0.8423	0.7297	1.15	0.248

- (iii) the number of tetrads occupied by both species in each year of each run;
- (iv) the mean duration that tetrads were occupied by both species was determined by calculating the duration that each tetrad contained both species of squirrel;
- (v) the predicted rate of decline in number of tetrad grid squares occupied by red squirrels was then calculated for each model run.

The impacts of contact rate and infection probability on the predicted rate of decline of red squirrels and the predicted mean duration of overlap by the two squirrel species in tetrads were investigated using linear regression.

#### Modelling the impacts of grey squirrel control in suppressing the decline of the red squirrel

Grey squirrel control is widely practised in Cumbria. We used the model to investigate what level of population control of grey squirrels would be necessary to suppress the disease-induced decline in the red squirrel populations in Cumbria. To achieve this we imposed control as a percentage kill of grey squirrels in all available habitats in which they were found, assuming this kill rate could be achieved at all sites with equal effectiveness in each of the years 1993–2003. We then estimated the effectiveness of control by predicting the number of tetrads in which red squirrel populations survived in 2003.

## RESULTS

### Observed changes in spatial distribution of red and grey squirrels and the incidence of SQPV disease

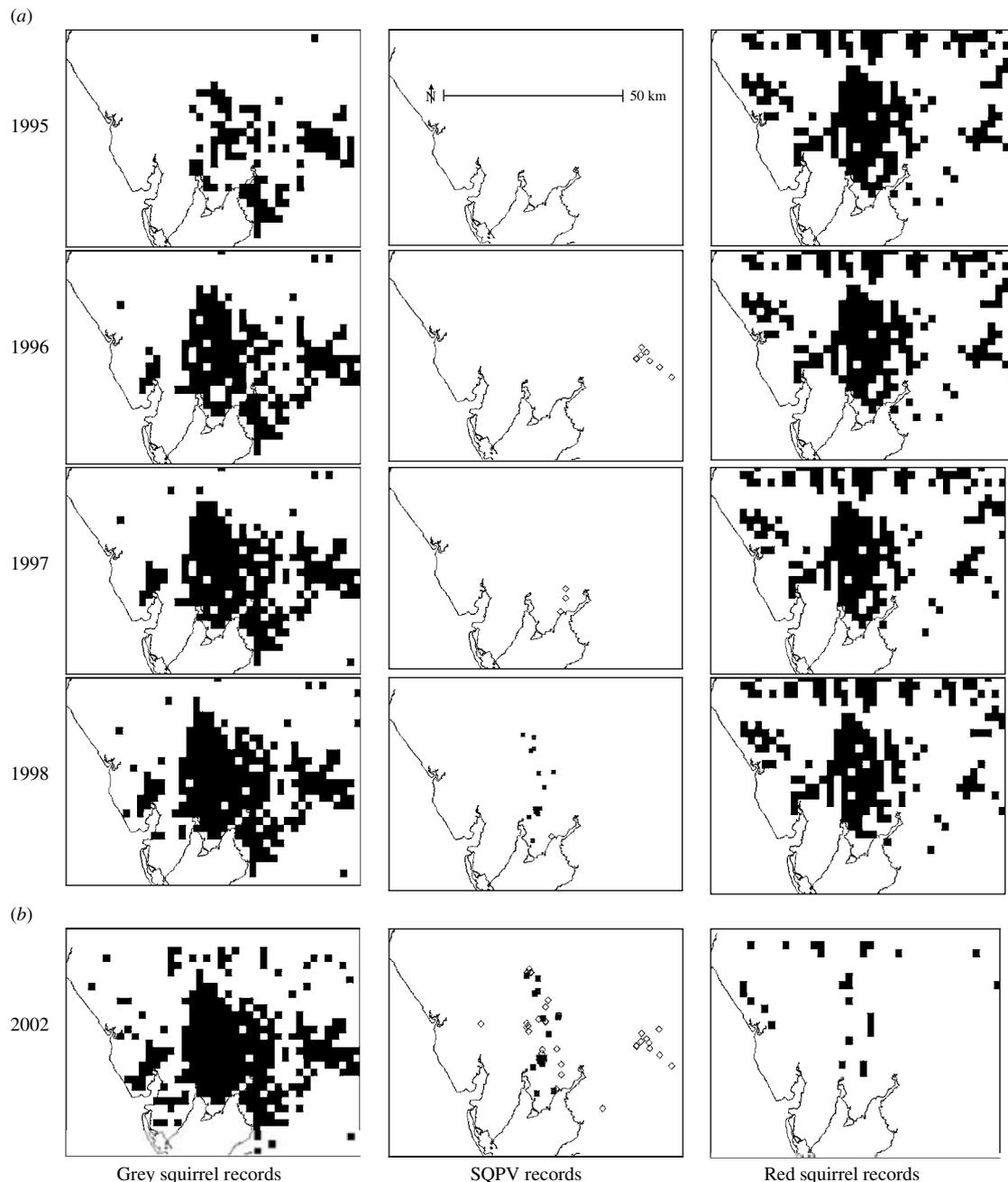
The observed pattern of spread of grey squirrels in Cumbria between 1993 and 2002 started from the south-east and moved through to the north-west (Fig. 1). This corresponds to grey squirrels colonizing Cumbria from Lancashire along woodlands between Morecambe Bay and the Pennines. Grey squirrel

spread was patchy at first followed by a consolidation and ‘filling in’. This was followed by disease outbreaks in red squirrels and a subsequent drastic red squirrel decline by 2002 (Fig. 1).

The observed rate of spread of grey squirrels was high from 1993 but slowed down after 1997 (Fig. 2*a*). The mean rate of expansion of grey squirrels over the study period was 119 km<sup>2</sup>/year. The distribution of red squirrels remained constant between 1993 and 1997 but had declined dramatically by 2002 (Fig. 2*b*). The mean rate of decline in red squirrel coverage from 1993 to 2002 was 76 km<sup>2</sup>/year. The number of tetrads in which both species were found increased from 1993 to 1997 and then decreased to a very low level by 2002. Squirrels were observed to coexist in 166 tetrads over the course of the 10 years of the study. The mean duration of overlap between the two species in tetrads was 3.31 years (S.D. = 1.96,  $n=166$ ). The duration of overlap (log transformed) was significantly related to the amount of deciduous woodland present in each tetrad ( $r=0.374$ ,  $P<0.000$ ; Table 4) but the variance in duration of overlap explained by this model was only 13%. There was no significant relationship between duration of overlap and the amount of conifer present in each tetrad.

There was a total of 27 records of red squirrels with SQPV disease over the period 1993–2003. The first was recorded in 1993 at the south-eastern end of the study area and 23 were recorded in 1998. Out of 183 grey squirrels tested, 99 were recorded as seropositive to SQPV over the same period (~50%). The greatest occurrence of seropositive grey squirrels was in 2000. The number of tetrads in which diseased red squirrels and seropositive grey squirrels were found was much less than the number of records when considered at the 100-m scale of recording suggesting that the occurrence of the disease organism in both species was clumped.

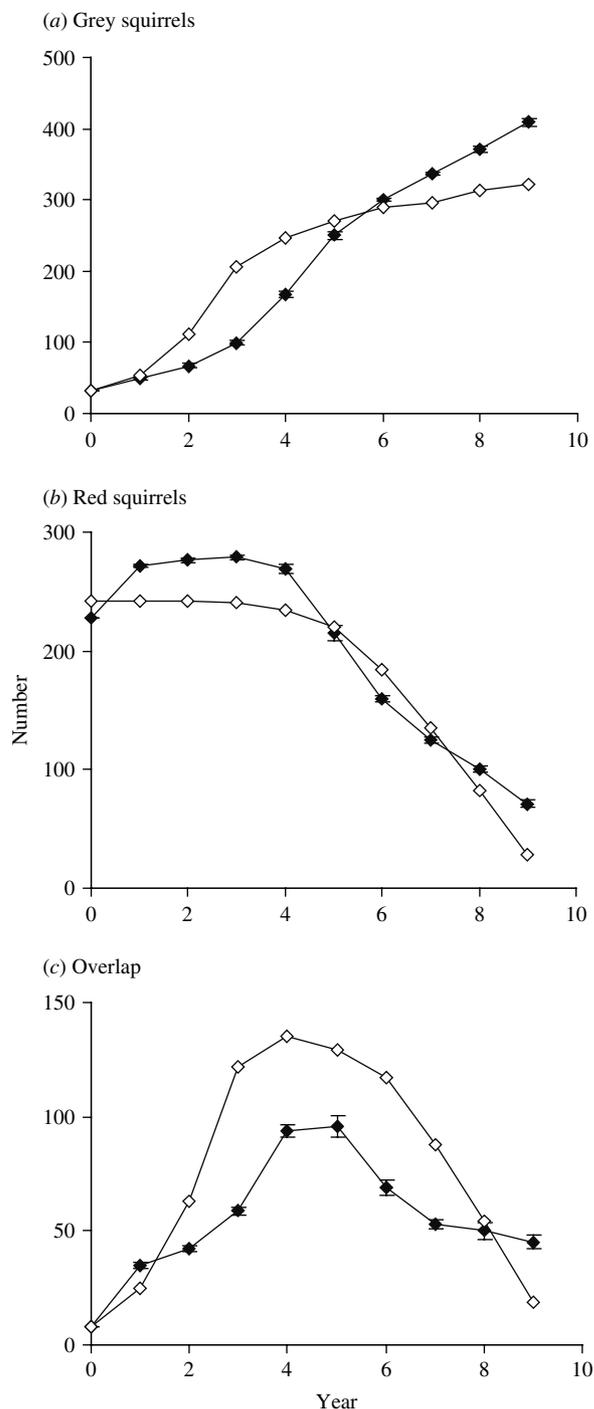
Of the 13 tetrads in which disease was recorded in red squirrels, grey squirrels were present in 11 at the time the disease was detected. Comparison of records



**Fig. 1.** Grey squirrel (left) and red squirrel (right) distribution records in south and central Cumbria as well as squirrel poxvirus (SQPV) records (centre) based on antibody testing in grey squirrels and diseased red squirrel records. (a) Shows the period for 1995–1998 and (b) the distribution in 2002. SQPV data illustration for 2002 (centre) represents a cumulative record over the period 1995–2002.

of SQPV disease in red squirrels with the distribution maps for grey squirrels over each year indicated that grey squirrels had been recorded in the same tetrad a mean of 3.1 (s.d. = 1.7) years prior to the detection of the disease. Grey squirrels seropositive to SQPV were observed in 32 tetrads of which 20 had red squirrels at the time of detection. Of the remaining 12 tetrads where seropositive animals were found, red squirrels

had been absent from between 0 and 6 (mean = 2.0, s.d. = 1.7) years prior to the detection of virus antibody in grey squirrels. A logistic regression relating occurrence of diseased red squirrels in tetrads to the area of available habitat, the duration of overlap in each tetrad and the presence of seropositive grey squirrels (Table 4) was significant for the first two predictors but not the presence of seropositive grey



**Fig. 2.** The number of tetrads in Cumbria observed to be occupied ( $\diamond$ ) and predicted ( $\blacklozenge$ ) by the model: (a) grey squirrels; (b) red squirrels and (c) both species over the period 1993–2002.

squirrels. These results indicate that the odds ratio for red squirrel disease to be recorded in a tetrad was 2.3 for every year of exposure to the presence of grey squirrels in the tetrad.

### Rates of decline in red squirrels in areas with and without SQPV

Rates of decline of red squirrel distribution in different regions of England, Scotland and Italy are shown in Table 5. The most obvious feature is that in Cumbria and Norfolk where we know SQPV to have been present, the decline in distribution was some 17–25 times faster than in Scotland and Italy where SQPV is known not to be present.

### Modelling the distribution of red and grey squirrel populations and SQPV infection from 1993 to 2003

We have only considered the analyses of the spread of grey squirrels for those model runs where grey squirrels were predicted to expand beyond the starting conditions representing the observed distribution for 1992. Where expansion by grey squirrels was successful, the MD of the predicted pattern from the observed pattern of spread of grey squirrels over the 10 years ranged from 42.3 to 192.9 tetrads/year (mean = 87.4, s.d. = 82.0,  $n = 129$ ). Four of these runs had values of  $< 50.0$  and were significantly different to the mean for the remainder ( $t = 8.71$ ,  $P < 0.0001$ ) indicating that the suite of life-history parameters led to a close match between observed and predicted spread of grey squirrels. The life-history inputs for these four model runs are shown in Table 2.

The MD of the predicted from the observed distributions of red squirrels for the 100 model runs with different sets of contact and infection probabilities ranged from 22.2 to 103.3 tetrads/year (mean = 59.0; s.d. = 31.7). For all runs where the MD was  $> 80.0$ , red squirrels did not decline. The log-transformed rate of decline in the number of tetrads occupied by red squirrels was significantly related to both the contact and infection probabilities used in the model runs (Table 6). Indicating that contact and infection probabilities both determined the extent to which the red squirrel population declined. The contact and infection probabilities for this run where the observed decline was closely matched by the model were 0.125 and 0.473 respectively. The mean period of coexistence of both species in tetrads as predicted by the model was significantly negatively related to both the contact probability and the infection probability used in the model (Table 7). This indicates, not surprisingly, that the duration of coexistence is predicted to be higher when contact and infection probabilities are low.

Table 5. Observed rates of red squirrel loss ( $\text{km}^2/\text{year}$ ) and habitat composition for areas with and without squirrel poxvirus present\*

	Cumbria	Norfolk	Italy	Scotland†
$\text{Km}^2$ lost/year	76	92	3.6	4.4
Pox present	Yes	Yes	No	No

\* Based on the following refs: [16, 34, 35].

† Based on areas that showed an increase in grey squirrel distribution [35].

A plot of the decline in red squirrels as predicted under this scenario and as observed in the field from 1993 to 2003 is shown in Figure 2*b*. For red squirrels the number of tetrads occupied stayed approximately constant at 240 from 1993 to 1997, after which the number of tetrads occupied declined linearly to <50 by 2002. For grey squirrels the expansion in tetrads occupied appeared to rise asymptotically, with the expansion declining after 1999. The predicted expansion of grey squirrels was slower than that observed and although there was an increased rate of expansion post-1999, the expansion did not appear to be approaching an asymptote. Changes in the number of tetrads occupied by both species over the same period are shown in Figure 2*c*. In this case both the predicted and observed numbers of tetrads occupied increased up to 1997 and 1998 before declining in 2002. The curve for the observed data was higher than that predicted by the model suggesting that the model under-predicted co-occupation of tetrads by the two species.

#### Modelling the impacts of grey squirrel control as a means of suppressing disease in red squirrels

The impacts of different levels of population control on the spread of grey squirrels and the decline of red squirrels in Cumbria as predicted by the model are shown in Figure 3. Where the kill rate was in excess of 60% grey squirrels were predicted not to spread into Cumbria, and red squirrel populations were predicted to be found in *about* 300 tetrads after 10 years. For any level of control below 60% grey squirrels were predicted to expand with a concomitant decline in red squirrel populations.

## DISCUSSION

Experimentation necessary to elucidate the role of disease in the mechanism of replacement of red

squirrels by grey squirrels together is practically difficult to undertake at an appropriate scale. We combined the study of patterns of decline in the field with modelling the underlying disease and population processes to assess the extent to which disease could have been an agent in the decline of the red squirrel and to evaluate if grey squirrel population control could mediate the decline. The success of this approach is contingent on the extent to which the current, limited data available could be used to represent the pattern of replacement and on the assumptions made in analysing these data.

The field data were not derived from a systematic survey of red and grey squirrel populations and associated disease, but the sporadic nature of disease epidemics and the large area covered would make it impossible to carry out such a survey. Moreover, both the spatial pattern of recording and the effort used varied through time. It is also possible that the distribution patterns for both species derived from the raw data exhibited bias. This bias is likely to have been different in the two squirrel species, as well as in the incidence of the disease. Red squirrels were not recorded in all tetrads in all years. The assumption that where squirrels were recorded in any tetrad in more than 3 years, then they were present in all years between may not have been true. This potential bias is probably less important than the assumption concerning the timing of when red squirrels became extinct in individual tetrads. Here, it was assumed that squirrels became extinct in a tetrad in the year that they were last recorded as being present. This will have inevitably led to bias in distribution maps, particularly if the effort and recorder intensity was lower in later years of the study. Red squirrels may have recolonized tetrads from which they were lost in the last 2 years of the sample period. In effect the decline in red squirrel distribution may have been exaggerated in the latter years of the study period a factor noted by Reynolds [16] in a study in Norfolk. It was also assumed that once a tetrad had been recorded as having grey squirrels present then they would be present in all subsequent years. This assumption may have led to an overestimation of the timing of the spread of grey squirrels, since there is evidence from some studies (but not all [36]) that individual grey squirrels appear 1 or 2 years ahead of the main body of individuals that establish the population [16, 37, 38]. Collectively and at worst, the spread of the grey squirrel, may have been overemphasized insofar as animals were recorded as being resident in

Table 6. Results of Generalized Linear Model relating the (log transformed) predicted rate of decline in tetrads (2-km grid squares) in Cumbria occupied by red squirrels to the probability of squirrel poxvirus infection and probability of contact between animals used in the simulation model ( $n=100$ )

	Estimate	s.d.	<i>t</i> value	Pr(>  <i>t</i>  )
(Constant)	-0.07244	0.22669	-0.320	0.75
Contact probability	9.04568	1.61731	5.593	$2.06 \times 10^{-7}$
Infection probability	2.91025	0.31009	9.385	$2.85 \times 10^{-15}$

Null deviance: 182.402 on 99 D.F.

Residual deviance: 73.716 on 97 D.F.; AIC 261.29.

Shapiro test for normality of residuals  $W=0.9767$ ,  $P=0.07324$ .

Table 7. Results of a Generalized Linear Model relating predicted mean duration of coexistence of red and grey squirrels in tetrads in Cumbria as predicted by the simulation model to the contact and infection rates used in the model ( $n=100$ )

Predictor	Coefficient	s.d.	<i>Z</i>	<i>P</i>
Constant	7.2175	0.1699	42.49	<0.001
Contact probability	-15.280	1.212	-12.61	<0.001
Infection probability	-3.7063	0.2323	-15.95	<0.001

places earlier than they actually were, and the decline of the red squirrel overestimated by missing animals that may have recolonized sites subsequently.

Bias in the virus infection dataset relates to the infection status of the two species and the methods of recording. For red squirrels the presence of poxvirus infection was confirmed by post mortem of diseased animals, whilst infection in grey squirrels was assessed by trapping individuals and carrying out a laboratory ELISA to determine the presence of SQPV antibodies [13]. Quantifying the impacts of these constraints is difficult but there can be little doubt that the occurrence of poxvirus in red squirrels was underestimated relative to the grey squirrel, since detection of the disease depended on corpses or sick animals being found and many animals will have died undetected. Nonetheless, given these constraints on the data, it is clear that the decline in red squirrels in Cumbria is linked to the expansion of grey squirrels, but does disease contribute to the speed of the decline?

Decline of red squirrels occurs in the presence of grey squirrels even when disease is known not to be

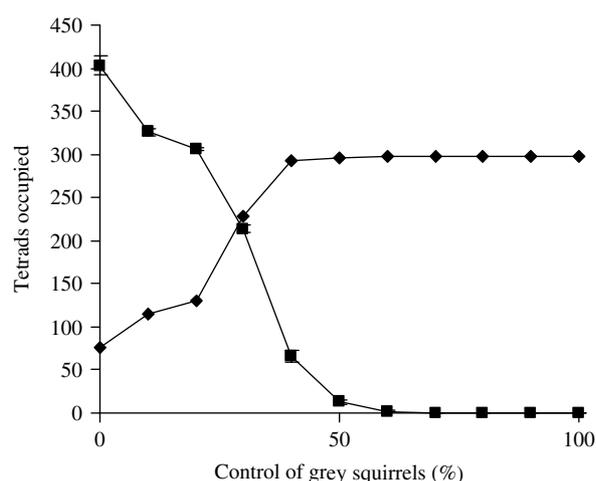


Fig. 3. The predicted effects of grey squirrel population control on grey squirrel colonization (—■—) and red squirrel decline (—◆—) in Cumbria. Control imposed as a percentage cull of grey squirrels in all habitats in which they are found. Note error bars for each plot are too small to discern on the plot.

present [34, 39]. Nonetheless, our comparison of regions with and without SQPV presence in the grey squirrel show that decline is 17–25 times faster where it is present. Whilst differences in habitat and landscape features between the regions are likely to have contributed to grey squirrel expansion and red squirrel decline, these results demonstrate that the presence of disease considerably enhances the rate of decline of red squirrels. The striking difference in the rates of decline suggest that disease far outweighs any impacts of inter-specific competition, but why is this so? The mechanism involved in inter-specific competition is a suppression of juvenile recruitment [3–5] with adult red squirrels remaining present in the landscape until they die. This mechanism is a slower

process than disease that acts more rapidly, once established, with high local mortality measured over weeks rather than years. This difference also explains why a previous attempt to model the decline in red squirrels in Norfolk [17] could find no discernible effect of inter-specific competition when modelled jointly with disease, since the disease process was modelled at the level of weeks whilst competition was assumed to occur on a yearly time step.

Rapid disease progression notwithstanding, the significant relationship between the first record of diseased red squirrels and the duration of overlap between the two species in Cumbria suggests that disease in red squirrels followed invasion by the grey squirrel and that the longer red squirrels were coexisting with this species the more likely they were to get the disease. This also implies that the appearance of grey squirrels in a tetrad did not mean that red squirrels succumb to the disease straightaway as suggested by Tompkins et al. [40]. It could be argued that the observed coexistence reflects the spatial scale at which both the field data and modelling analyses were undertaken. Squirrels might have been able to occupy different habitat blocks within the same tetrad and hence may not have come into close contact for 2–3 years. This would significantly influence encounter rates and transmission between the two species. The significant relationship between the duration of overlap and the amount of deciduous woodland from the field data gives some support for this argument, although the amount of variation explained by habitat in this model was small (13%). It is more likely, however, that other population and disease-transmission processes determine the overlap period. First, the model results suggest that only under comparatively low contact and transmission probabilities will disease cause decline in red squirrels similar to that observed in the field. Previous research has demonstrated the robustness of spatially explicit models for simulating the demographics of both red and grey squirrels [17, 18, 30, 39], suggesting that the low transmission and infection rates needed to cause the observed decline are realistic. The current model did not include separate contact and infection probabilities for each species simply because we had no data and to have included separate variables would have doubled the number of unknowns within the model. The low contact and transmission probabilities required to generate the observed pattern of red squirrel decline therefore reflect confounding with and between species behaviours as well as potential

errors arising from non-inclusion of inter-specific competition. Nonetheless, both species are solitary and, although they may feed amicably in the same tree or occasionally use the same dreys (nests) at different times, direct contact between the species is likely to be low [5, 41, 42]. It is also possible that this apparent overlap reflects variation in the seroprevalence status of grey squirrels. Local differences [13] could have influenced both the likelihood of disease transmission and the encounter rates between and within species. Spatial heterogeneity in population structure is known to influence disease spread within species [43]. Under circumstances of low SQPV prevalence in grey squirrels, inter-specific competition might become the dominant process in the replacement of red by grey squirrels, effectively extending the period of apparent coexistence.

What are the implications of our results for the management of red and grey squirrel populations? The results of the modelling suggests that a sustained strategy of control with a 60% kill during the invasion period from 1993 to 2003 could have suppressed the decline in red squirrels in Cumbria. This is in agreement with the results that showed that grey squirrel population control to ensure red squirrel population survival was theoretically feasible (but expensive) at an invasion front similar to that faced by Cumbria in 1993 [31]. Unfortunately the distribution of the grey squirrel has expanded dramatically since 1993. Furthermore, although Gurnell & Pepper [44] suggested a regional defence strategy as part of the armoury to conserve red squirrels, present control strategies are not designed for invasion fronts. Currently, grey squirrel control for red squirrel conservation is used as a short- to medium-term tactic to reduce grey squirrels numbers as much as possible in forests where the two species coexist, thus reducing the competitive effect of the greys on reds [44]. It is unlikely that such a strategy would be useful where disease is the agent responsible for the decline, and control strategies need to be re-designed to cater for landscape level consideration of invasion fronts, and targeting to minimize grey squirrel dispersal and contact rates between the two species. This has implications for the spatial application of control measures, their timing and their cost.

The current findings show the role of SQPV in the decline of the red squirrel population in northern England. The impending spread of the disease into Scottish grey squirrel populations in the Solway area,

Cumbria leads us to conclude that the outlook for red squirrels in Scotland is bleak without a clear policy on the control of grey squirrels and SQPV containment.

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#### DECLARATION OF INTEREST

None.

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