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RESEARCH ARTICLE

Immune interactions and heterogeneity in transmission drives the pathogen-mediated invasion of grey squirrels in the UK

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Abstract

1. Mathematical models highlighted the importance of pathogen-mediated invasion, with the replacement of red squirrels by squirrelpox virus (SQPV) carrying grey squirrels in the UK, a well-known example.
2. In this study, we combine new epidemiological models, with a range of infection characteristics, with recent longitudinal field and experimental studies on the SQPV dynamics in red and grey squirrel populations to better infer the mechanistic basis of the disease interaction.
3. A key finding is that a model with either partial immunity or waning immunity and reinfection, where individuals become seropositive on the second exposure to infection, that up to now has been shown in experimental data only, can capture the key aspects of the field study observations.
4. By fitting to SQPV epidemic observations in isolated red squirrel populations, we can infer that SQPV transmission between red squirrels is significantly (4×) higher than the transmission between grey squirrels and as a result our model shows that disease-mediated replacement of red squirrels by greys is considerably more rapid than replacement in the absence of SQPV.
5. Our findings recover the key results of the previous model studies, which highlights the value of simple strategic models that are appropriate when there are limited data, but also emphasise the likely complexity of immune interactions in wildlife disease and how models can help infer disease processes from field data.

KEYWORDS
conservation, epidemiological modelling, invasive species, shared pathogen

1 | INTRODUCTION

Pathogens that threaten endangered wildlife species are typically transmitted from reservoir hosts (Daszak et al., 2000), and disease-mediated invasion has been shown to be a key driver of native species replacement (Hatcher et al., 2006; Prenter et al., 2004; Strauss et al., 2012). Therefore, understanding both the epidemiological dynamics and the disease processes that determine the persistence of infection in reservoir hosts and their outbreak dynamics in the endangered species are critical in determining conservation strategies (Viana et al., 2014). An important case study example of disease-mediated invasion is the transmission of squirrelpox virus (SQPV), from its reservoir host, grey squirrels (*Sciurus carolinensis*) to endangered red squirrels (*Sciurus vulgaris*), in the British

Isles (Sainsbury et al., 2000, 2008; Tompkins et al., 2003 and see Wauters et al. (2023) for a comprehensive review of grey squirrel invasion). Grey squirrels are an invasive species in the British Isles, which were introduced from North America in the late 19th century (Middleton, 1930) most probably along with its natural pathogen SQPV (McInnes et al., 2006). Squirrelpox virus has low virulence in grey squirrels and persists at high prevalence in established grey squirrel populations (Chantrey et al., 2019; Sainsbury et al., 2000) but can spillover to red squirrels and can cause epidemic outbreaks of disease in red squirrels that can reduce population density by up to 90% (Chantrey et al., 2014). In combination with field observations (Carroll et al., 2009; Sainsbury et al., 2008), and beginning 20 years ago (Tompkins et al., 2003), mathematical models have played a key role in understanding the role of SQPV in the replacement of red squirrels by greys (Rushton et al., 2006; Tompkins et al., 2003) and the spread of SQPV through established grey squirrel populations (White et al., 2014, 2016). Due to a lack of data on the disease processes at the time, these model frameworks assumed, for generality, classical, simple epidemiological dynamics: for red squirrels, the models assumed infection without recovery (a susceptible-infected, SI model framework), and for grey squirrels, they assumed recovery to life-long and complete immunity (a susceptible-infected-recovered/immune, SIR model framework). With these assumptions, SQPV could be shown to persist with seroprevalence levels that are consistent with field observations (Tompkins et al., 2003; White et al., 2016) and importantly showed that the presence of SPQV would lead to the more rapid replacement of red squirrels by greys squirrels, with the key result that the presence of SQPV was required for replacement times to match field studies (Reynolds, 1985; Tompkins et al., 2003). These simple models highlighted the potential importance of the virus in the replacement of red squirrels, leading to the use of more focussed models to develop conservation strategies (White et al., 2014, 2016) and importantly, stimulating the collection of experimental (Fiegna, 2012) and field data (Chantrey et al., 2014, 2019).

New field and experimental data now allow the model framework and parameters used by Tompkins et al. (2003) to be refined and updated. First, Chantrey et al. (2014) recorded SQPV epidemiological dynamics in isolated red squirrel populations in Formby, Merseyside, where an SQPV outbreak reduced red squirrel density by 90%. We can use the data to estimate SQPV transmission rates between red squirrels. Second, a longitudinal study of grey squirrel viraemic and serological status indicated that grey squirrels can become reinfected with SQPV (Chantrey et al., 2019), and furthermore, their results found SQPV infection to occur at a high prevalence, with repeated periods of recovery and reinfection despite a concurrent antibody response. The data (Chantrey et al., 2019) show that the percentage of viraemic/infective grey individuals would be 25%, an order of magnitude higher than the endemic levels reported in Tompkins et al. (2003), and furthermore, they found no link between antibody levels and presence or absence of the virus, suggesting that the antibody response may be relatively ineffective at controlling the virus (Chantrey et al., 2019). They speculate that individual squirrels may either clear the infection but become reinfected, or that the infection may remain at low levels, with bouts of recrudescence (although they acknowledge that recrudescence has not been reported in other poxvirus infections). The densities and demographics of the grey squirrel populations are likely to be comparable with wider national levels, and therefore, the infection dynamics that they report should be representative of those in grey squirrel population in the UK (Chantrey et al., 2019). There are also limited experimental data (Fiegna, 2012) that suggest that seroconversion is rare at first infection but may occur readily after a second challenge. Taken together, these data allow us to build models to infer the likely fundamental disease processes in this system and then determine the role of the virus in red squirrel replacement.

In this study, we aim to use mathematical models, combined with field study and experimental data, to gain a better understanding of the epidemiological dynamics of SQPV in red and grey squirrels. We will combine the SI model framework proposed by Tompkins et al. (2003) for the infection dynamics in red squirrels and the data of Chantrey et al. (2014) to estimate SQPV transmission within red squirrel populations. We will develop a suite of mathematical models to explore the outcome of different assumptions about the immune response, and reinfection or recrudescence mechanisims, on the epidemiological dynamics of SQPV in grey squirrels. Our aim will be to understand which infection mechanisms can reproduce the viraemic and seropositive population levels observed in the study of Chantrey et al. (2019). We match average properties from the Chantrey et al. (2019) study—seroprevalence, percentage viraemic, time between infection and maximum infection duration—with the equivalent average properties in the mathematical frameworks. Our model frameworks are tailored to represent the red and grey squirrel population and epidemiological dynamics but are also strategic, general, frameworks and therefore fitting to average properties from the data is appropriate. A better understanding of the dynamics of SQPV in red and grey squirrels will allow us to better infer transmission risk and the impact of SQPV on the endangered red squirrel. The insight we gain from the new models is used to review the current understanding of red squirrel conservation when threatened with invasion from SQPV carrying grey squirrels. We show how mathematical models can help understand and interpret epidemiological processes from field study observations and also allow us to better predict the epidemiological impact of shared pathogens on important wildlife systems that are a conservation concern.

2 | MATERIALS AND METHODS

2.1 | Supporting empirical data

We draw on three key empirical studies. Chantrey et al. (2014) collected data between 2002 and 2012 on SQPV infection levels and the abundance of red squirrels before, during and after a SQPV epidemic outbreak in Formby, Merseyside, UK. As such, the data give us information on an outbreak epidemic of the disease in a red

squirrel population. Chantrey et al. (2019) carried out a longitudinal study that trapped, marked, sampled and released grey squirrels at monthly intervals between August 2010 and December 2011 in Ness Botanical Gardens (UK) where grey squirrels persist and there are no neighbouring red squirrel populations (available as supplementary data in Chantrey et al., 2019). ELISA was used to detect SQPV antibodies in blood sera, cutaneous/mucocutaneous swabs were used to determine the infection/shedding status of SQPV and tested for the presence of viral DNA. This provides a time series of the SQPV infectious status and antibody status for individual squirrels over the study period (although an individual may avoid capture in a particular month and so there are gaps in the time series). Results were presented for the proportion of individuals that tested positive for SQPV viraemia and SQPV antibodies. As such the data gave us information on the immune and viral dynamics of the endemic disease in grey squirrel populations. Finally, Fiegna (2012) experimentally infected captive grey squirrels and used ELISA on blood sera to test for the presence of antibodies against SQPV over a period of 14 weeks. As such, this gives us information on the immune response in individual grey squirrels. Our models allow us to gain insights into the epidemiology of the SQPV interaction using these diverse data sources from different scales.

Specifically, we use the data of Chantrey et al. (2014) at sites E & F. These sites are red squirrel strongholds where red squirrels are protected and grey squirrels are excluded through targeted control. However, control cannot prevent the periodic transfer of infection between species at the interface between red and grey squirrel populations (White et al., 2014). The average density of red squirrels at the two sites in the absence of infection was 90/km^2. An SQPV outbreak occurred in 2008, resulting in a 90% decrease in red squirrel population density, followed by SQPV fade-out and a return to the pre-infection density for the red squirrel populations.

We use the data of Chantrey et al. (2019) and determine the mean (±95% confidence intervals) percentage of seropositive grey squirrels, 80 ± 3.6%, and the mean percentage of viraemic individuals, 25 ± 4.0%. The data of Chantrey et al. (2019) also showed that once an individual is observed to be seropositive, they remain seropositive for the subsequent duration of the study period. This indicates that antibodies to SQPV may only provide partial immunity, or that immunity may wane, leading to reinfection. We further analyse the data of Chantrey et al. (2019) to provide estimates of epidemiological characteristics that can be compared with the results of our models. To determine an estimate of the average maximum infection period, we used data from individuals observed to be infected at least once (60 samples) and calculated the difference between the latest date at which individuals were observed to be non-viraemic, plus one day, and the next date at which they were observed to be non-viraemic, minus one day. The average maximum infection period was 6.8 ± 1.0 weeks. To determine an average time between infections, we combined two methods. For individuals who were infected multiple times over the study, with a non-viraemic observation between viraemic recordings, we calculated the time between viraemic observations (33 samples) with average 14.0 ± 3.08 weeks between infections. We also calculated the time between the start of the study and the first viraemic observation and the final viraemic observation and the end of the study. When this period was >14.0 weeks (32 samples), we combined the data with the data for the time between two viraemic observations to provide an estimate of the mean time between infections of 19.1 ± 3.17 weeks.

Fiegna (2012) showed that out of 38 individuals, only 8% of grey squirrels challenged with SQPV inoculum showed an antibody response within 3 weeks. Three individuals were challenged a second time after 10 weeks, which led to two individuals testing seropositive to SQPV after 1 week, and all three seropositive after 3 weeks. The data suggested that individuals may typically become seropositive to SQPV after a second challenge from the virus. Furthermore, the data of Chantrey et al. (2019) support this observation showing evidence of instances where individuals remain seronegative on recovery from positive viraemia.

2.2 | Mathematical modelling overview

We redefine and extend the model framework that represent the population and epidemiological dynamics of red and grey squirrels as outlined by Tompkins et al. (2003). In their study, red squirrel epidemiological dynamics were represented by an SI model as SQPV infection is generally fatal for red squirrels with little evidence of acquired immunity (Chantrey et al., 2014; Tompkins et al., 2002). Grey squirrel epidemiological dynamics were represented by an SIR model, as grey squirrels show antibodies to SQPV, and it was assumed that they recover to life-long and complete immunity. As in Tompkins et al. (2003), we assume the death rate is constant and the birth rate is density-dependent and modified by intra- and interspecific competition to reflect that competition for resources can reduce reproductive levels (Wauters et al., 2000). This formulation for the demographic processes has been used to represent the UK squirrel system (Jones et al., 2017; Macpherson et al., 2016; Slade et al., 2020; White et al., 2014), but we acknowledge that other model formulations that include density-dependent growth (Barbara et al., 2018; Okubo et al., 1989), or death (Roberts & Heesterbeek, 2021) have also been used and report similar findings (see Wauters et al., 2023). In Tompkins et al. (2003), the demographic parameters, the rate of disease-induced mortality in red squirrels and the rate of recovery from infection for grey squirrels were estimated from field and laboratory studies. The SQPV transmission coefficient between grey squirrels was determined by matching the model results with field observations that indicated that the average grey squirrel seroprevelence for SQPV in study populations in England and Wales was 74% (Sainsbury et al., 2000). Squirrelpox virus transmission between red squirrels, and from red to grey and grey to red squirrels, was assumed to be the same as that estimated between grey squirrels.

In this study, we retain the SI model framework to represent the epidemiological dynamics of red squirrels, as suggested by data (Chantrey et al., 2014; Tompkins et al., 2002), but use the data of Chantrey et al. (2014) to determine an independent estimate of the

transmission of SQPV between red squirrels. We consider several model frameworks of the grey squirrel SQPV system in which grey squirrels can be reinfected, as observed in Chantrey et al. (2019). Our models are extensions of the SIR compartmental model of Tompkins et al. (2003). We consider

(i) a straightforward extension of the SIR framework of Tompkins et al. (2003) where instead of recovery from infection conveying life-long immunity we assume it conveys partial immunity. Here recovery from infection leads to a reduction in the chance of further infection;
(ii) an extension of the framework in (i) to account for the observation that individuals may not become seropositive until their second instance of infection (Fiegna, 2012).

In the supplementary information we present two further potential models for grey squirrels

(i) we assume waning immunity, where recovery confers full immunity but this wanes over time and an individual becomes 'fully' susceptible once again; and
(ii) we consider a model of recrudescence for the grey squirrel infection dynamics, a mechanism suggested by Chantrey et al. (2019) in which recovered individuals can revert to infected individuals without the need for a 'new' contact with an infected individual.

We test whether the different model frameworks, and the epidemiological processes that they represent, can capture the observed proportions of viraemic and seropositive individuals, and the observed duration of infection and time between infection, for parameters that reflect grey squirrel demographics (with the demographic parameters taken from Tompkins et al., 2003).

3 | MATHEMATICAL MODELS AND RESULTS

3.1 | Estimating SQPV transmission between red squirrels

We assume an SI model framework for the epidemiological dynamics of red squirrels that represents the density of susceptible, S_R, and infected, I_R, individuals and can be modelled by the following equations:

$$\begin{aligned}\frac{dS_R}{dt} &= (a_R - q_R H_R)H_R - \beta_R S_R I_R - b_R S_R \\ \frac{dI_R}{dt} &= \beta_R S_R I_R - b_R I - \alpha I_R\end{aligned} \tag{1}$$

where $H_R = S_R + I_R$. The parameter $a_R = 1.0$/year is the maximum birth rate, which is modified by competition for resources with coefficient q_R (km^2/year), $b_R = 0.4$/year is the natural death rate, and $\alpha = 26$/year is the disease-induced rate of mortality (all parameter values are taken from Tompkins et al. (2003)). We assume that the carrying capacity $K_R = 90$/km^2 for red squirrels to match the average density at sites E & F in Chantrey et al. (2014) and this defines $q_R = (a_R - b_R)/K_R$. When infection is introduced into the model system there is an initial outbreak that reduces the population to a minimum level, before the density increases and tends to an endemic equilibrium. We solve Equation 1 numerically assuming that two infected red individuals are introduced into a population of red squirrels at their carrying capacity, K_R, for different values of the SQPV transmission coefficient, β_R, and determine the minimum value of β_R that leads to a 90% reduction in the population density level following an SQPV outbreak. We estimate that the SQPV transmission coefficient between red squirrels, $\beta_R = 0.82$km^2/year, meaning that a threshold density of 32 red squirrels/km^2 is required to support SQPV. This value is similar to the estimate used by Tompkins et al. (2003) of $\beta_R = 0.7$km^2/year (threshold density of 38 red squirrels/km^2) and importantly significantly higher that the SQPV transmission coefficient between grey squirrels predicted in this study (see Section 3.3).

3.2 | Grey squirrel epidemiological dynamics: Partial immunity and reinfection

We extend the SIR model framework for the grey squirrel epidemiological dynamics of Tompkins et al. (2003) to allow recovered individuals to become reinfected. Our model framework represents the dynamics of grey squirrels where S is the density of susceptible individuals that are vulnerable to infection, I is the density of viraemic/infected individuals that can transmit the virus and R the density of recovered individuals, who have a reduced susceptibility to infection (partial immunity). The dynamics are represented as follows:

$$\begin{aligned}\frac{dS}{dt} &= (a_G - q_G H)H - \beta_G SI - b_G S \\ \frac{dI}{dt} &= \beta_G SI + \sigma\beta_G RI - b_G I - \gamma I \\ \frac{dR}{dt} &= \gamma I - \sigma\beta_G RI - b_G R\end{aligned} \tag{2}$$

where $H = S + I + R$, $a_G = 1.2$/year is the maximum birth rate, which is modified due to competition for resources with coefficient, $q_G = 0.01$ km^2/year and $b_G = 0.4$/year is the natural death rate. The demographic parameters are taken from Tompkins et al. (2003) and assume the carrying capacity $K_G = (a_G - b_G)/q_G = 80$/km^2 which is representative of a mixed, mostly broadleaf forest habitat (Slade et al., 2020) and of the Ness Gardens study site. We assume infection occurs due to direct contact with transmission coefficient β_G (/year). Infected individuals recover from infection to enter the recovered class at rate γ (/year) with the quantity $1/\gamma$ representing the average duration of infection. Immunity is partial therefore recovered individuals can be reinfected, but with a reduced transmission coefficient $\sigma\beta_G$ where $\sigma \in (0, 1)$. The endemic steady state of the system represented by Equation 2 is $(S, I, R) = (S^*, I^*, R^*)$, and this is positive and stable if $R_0^G > 1$ where

$$R_0^G = \frac{\beta_G K_G}{b_G + \gamma} \tag{3}$$

Using the values at the endemic steady state, we define the proportion of the population that is seropositive as $\frac{I^*+R^*}{H^*}$ and set this equal to $P = 0.8$ (the average seroprevalence level in Chantrey et al., 2019, see also Section 2). We can determine an explicit expression for the infection transmission coefficient, β_G, as:

$$\beta_G = \frac{q_G\left(((\sigma-1)b_G-\gamma)P+b_G+\gamma\right)}{(a_G-b_G)(1+(\sigma-1)P)(1-P)} \tag{4}$$

Equation 4 ensures that the proportion of seropositive individuals is 80% for different combination of the parameters γ and σ. We then assess whether the model can represent the observed proportion of viraemic individuals (I^*/H^*) of 0.25 (reported by Chantrey et al., 2019, see Section 2).

Figure 1a indicates that for the proportion of viraemic individuals to be 0.25, it requires $\sigma \approx 1$, indicating little or no partial immunity, and $\gamma < 5$/year equating to an average infectious period greater than 10 weeks, considerably higher than the average maximum infectious period predicted by the data (Chantrey et al. (2019), see Section 2). Our model results therefore suggest that the process of partial immunity and reinfection, in which individuals are seropositive from the time that they are first infected, does not lead to the observed viraemic and seropositive levels reported by Chantrey et al. (2019), while also reflecting the duration of infection from the field data. Note, the findings are similar for an equivalent model of waning immunity (see Supporting Information Section S.1).

3.3 | Grey squirrel epidemiological dynamics: Partial immunity and reinfection with seropositivity after multiple infections

We extend the model of reinfection represented by Equation 2 by introducing additional classes that can track whether an individual is infected for the first time or for the second and subsequent times. The model framework is as follows:

$$\begin{aligned}
\frac{dS_1}{dt} &= (a_G - q_G H)H - \beta_G S_1(I_1+I_A) - b_G S_1\\
\frac{dI_1}{dt} &= \beta_G S_1(I_1+I_A) - \gamma I_1 - b_G I_1\\
\frac{dS_2}{dt} &= \gamma I_1 - \beta_G S_2(I_1+I_A) - b_G S_2\\
\frac{dI_A}{dt} &= \beta_G S_2(I_1+I_A) + \sigma\beta_G R_A(I_1+I_A) - \gamma I_A - b_G I_A\\
\frac{dR_A}{dt} &= \gamma I_A - \sigma\beta_G R_A(I_1+I_A) - b_G R_A
\end{aligned} \tag{5}$$

Here, $H = S_1 + I_1 + S_2 + I_A + R_A$, I_1 represents the initial infection, and I_A represents the second and subsequent infections during which individuals are seropositive. We assume individuals are fully susceptible prior to initial infection (S_1) and following recovery from the first infection (S_2) but have partial immunity following subsequent infection (R_A). Here the subscript A indicates individuals are antibody positive (seropositive). The endemic steady state of the system represented by Equation 5 defined as $(S_1, I_1, S_2, I_A, R_A) = (S_1^*, I_1^*, S_2^*, I_A^*, R_A^*)$, is positive and stable if $R_0^G > 1$ where R_0^G is the same as in Equation 3. This system is more complex

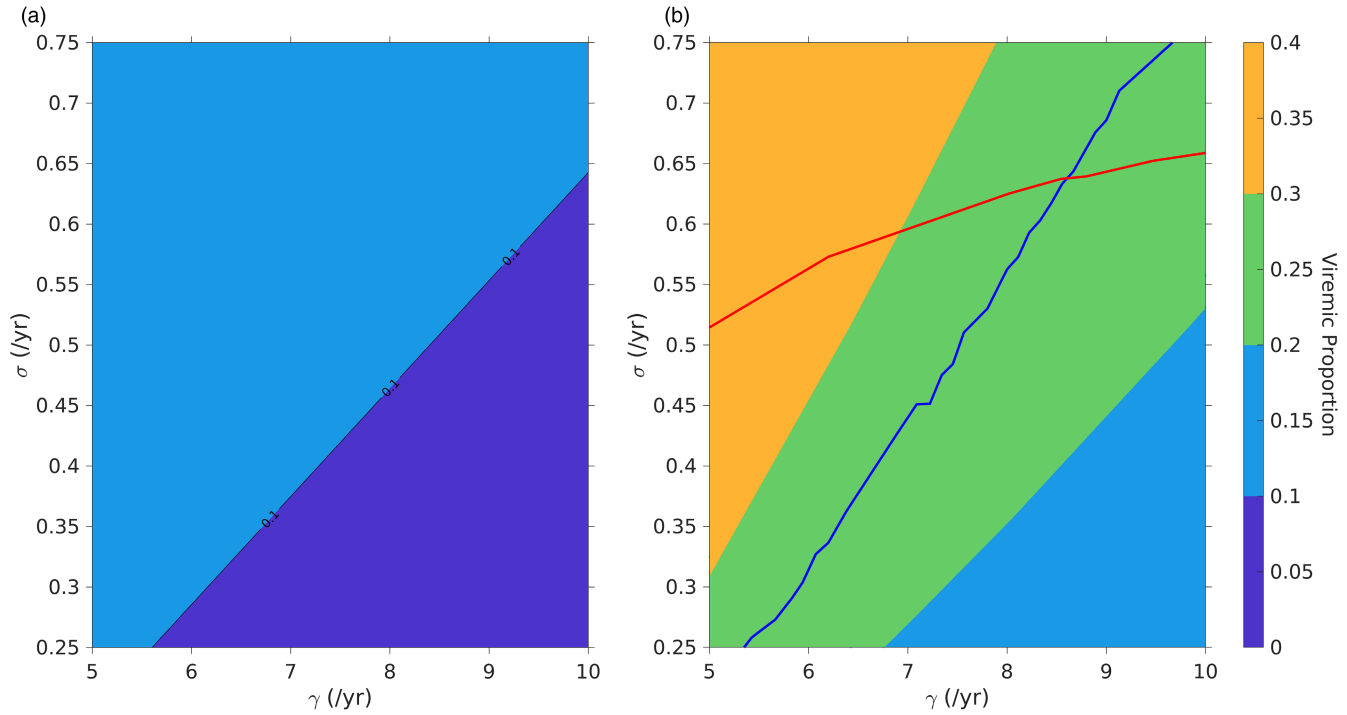

FIGURE 1 Proportion the population that is viraemic plotted against γ and σ. (a) shows the results for the model with partial immunity and reinfection (Equation 2), and (b) shows the results for the model with partial immunity and reinfection, with seropositivity on reinfection (Equation 5). In (b) the blue line indicates where the proportion of the population that is viraemic is 0.25, the red line indicates where the time between infection, defined as $1/(\sigma\beta_G(I_1+I_A))$, is 19 weeks. Panel (a) is plotted using Equation 4 while (b) is plotted numerically (which explains the why the red and blue lines are not smooth).

and finding an explicit algebraic expression for β_G is not possible, instead we solve the system of Equation (5) numerically to find the value of β_G for a range of γ and σ values that lead to a seroprevalence level of 80% ($(I_A^* + R_A^*)/H^* = 0.8$). For each of these values we plot the viraemic percentage $(I_1^* + I_A^*)/H^*$ as shown in Figure 1b. For this model set-up, there are a range of values of γ and σ for which the viraemic proportion is 25%, and in particular this condition can be satisfied for parameter combinations in which the average infectious period is less that 6.8 weeks ($\gamma > 7.65$/year). This indicates that an epidemiological framework with partial immunity and an antibody response on reinfection could fit the field data. We use this model to determine a set of baseline parameters where the percentage of infected is 25% and the time between infection is 19 weeks (as estimated from Chantrey et al., 2019, see Section 2). The baseline infection parameters are $\gamma = 8.7$/year, $\sigma = 0.62$, $\beta_G = 0.22\text{km}^2$/year and we use these to explore the implications of the epidemiological framework represented by Equation (5) on the interaction between grey and red squirrels (see Section 3.4). We note that the SQPV transmission between red squirrels ($\beta_R = 0.82\text{km}^2$/year) is predicted to be nearly four times greater than that between grey squirrels ($\beta_G = 0.22\text{km}^2$/year).

A model with waning immunity (see Supporting Information Section S.1) with baseline parameters $\gamma = 8.6$/year, $\nu = 7.3$/year, $\beta_G = 0.22\text{km}^2$/year could also fit the field data. The model results and findings that follow are similar for the model of partial and waning immunity and suggests that these mechanisms lead to similar dynamics in our model frameworks. A model of recrudescence, as suggested by Chantrey et al. (2014, see Supporting Information Section S.2) with baseline parameters to fit the proportions required ($\gamma = 6.90$/year, $\omega = 2.73$/year, $\beta_G = 0.08\text{km}^2$/year) did not fit the field data, requiring an average infectious period in excess of the maximum calculated for the observed data.

3.4 | Disease-mediated replacement of red squirrels

The SQPV dynamics in grey squirrels in the model proposed by Tompkins et al. (2003), that included life-long immunity following infection, supported an infected population of approximately 2.5% of the total population. In our model (Equation 5) that represents the observations of Chantrey et al. (2019), and includes reinfection due partial immunity, the infected population density is considerable higher, representing 25% of the total population. This may have a considerable impact on the role of grey squirrels in the disease-mediated replacement of red squirrels. To examine this impact, we combine the model of SQPV epidemiology in red squirrels (Equation 1) with the the model of SQPV epidemiology for grey squirrels (Equation 5), that can represent the observations of Chantrey et al. (2019), to include the competitive and infection interactions between red and grey squirrels. The model is as follows:

$$\begin{aligned}
\frac{dS_1}{dt} &= \left(a_G - q_G\left(H_G + c_R H_R\right)\right)H_G - \beta_G S_1\left(I_1 + I_A\right) - \beta_R S_1 I_R - b_G S_1\\
\frac{dI_1}{dt} &= \beta_G S_1\left(I_1 + I_A\right) + \beta_R S_1 I_R - b_G I_1 - \gamma I_1\\
\frac{dS_2}{dt} &= \gamma I_1 - \beta_G S_2\left(I_1 + I_A\right) - \beta_R S_2 I_R - b_G S_2\\
\frac{dI_A}{dt} &= \beta_G S_2\left(I_1 + I_A\right) + \beta_R S_2 I_R + \sigma\beta_G R_A\left(I_1 + I_A\right) + \sigma\beta_R R_A I_R - \gamma I_A - b_G I_A\\
\frac{dR_A}{dt} &= \gamma I_A - \sigma\beta_G R_A\left(I_1 + I_A\right) - \sigma\beta_R R_A I_R - b_G R_A\\
\frac{dS_R}{dt} &= \left(a_R - q_R\left(H_R + c_G H_G\right)\right)H_R - \beta_G S_R\left(I_1 + I_A\right) - \beta_R S_R I_R - b_R S_R\\
\frac{dI_R}{dt} &= \beta_G S_R\left(I_1 + I_A\right) + \beta_R S_R I_R - b_R I_R - \alpha I_R
\end{aligned} \tag{6}$$

where $H_G = S_1 + I_1 + S_2 + I_A + R_A$, and $H_R = S_R + I_R$. To match the study of Tompkins et al. (2003), we assume the carrying capacity of red squirrels is $K_R = 60/\text{km}^2$ (and this modifies q_R). We assume competition between red and grey squirrels that acts to modify the birth rate, with $c_R = 0.61$ and $c_G = 1.6$ representing the competition from red and grey squirrels on the other species, respectively. Red and grey squirrels can become infected through contact with infected individuals of either species, with transmission coefficients β_G and β_R respectively. Here, we are assuming that the infectivity of red squirrels is greater than the infectivity of grey squirrels. There is evidence for this as infected red squirrels display more open wounds (largely absent in infected grey squirrels) and these lesions may be a key mechanism for shedding the virus and thus spreading infection (Fiegna, 2012). The remaining red and grey squirrel parameters are as described for Equations 1 and 5. Figure 2a,b shows the results from the original Tompkins et al. (2003) model in which recovery from SQPV for grey squirrels leads to life-long immunity. Here, when greys replace red squirrels due to competition (Figure 2a) the replacement time is approximately 15 years, whereas when replacement is due to disease-mediated competition (Figure 2b) the replacement time is reduced to approximately 7 years. Figure 2c shows the results for Equation 6 where SQPV infected grey squirrels are introduced to a red squirrel population at its carrying capacity (note if uninfected grey squirrels are introduced the results are the same as in 2a). This shows that SQPV infection carried by grey squirrels can lead to an initial epidemic outbreak in red squirrel populations and that the rate of replacement of red squirrels by grey squirrels is increased when SQPV is present compared to when it is absent. Our model assumes that SQPV infectivity of red squirrels would be approximately four times higher than that of grey squirrels and this can lead to the more rapid replacement of red squirrels when SQPV is present. We also tested other transmission assumptions where we assumed red squirrel susceptibility to SQPV may be the cause of increased transmission between red squirrels and where red squirrels may have increased infectivity and susceptibility (see Supporting Information Section S.3). This indicated that the key driver of disease-mediated replacement was the rate of transmission between red squirrels (see Supporting Information, Figure S.3). Under both our model set-up and Tompkins et al. (2003) transmission from infected grey squirrels to red squirrels causes an outbreak of SPQV in red squirrels and a population crash. As, initially, grey squirrels are at low density this

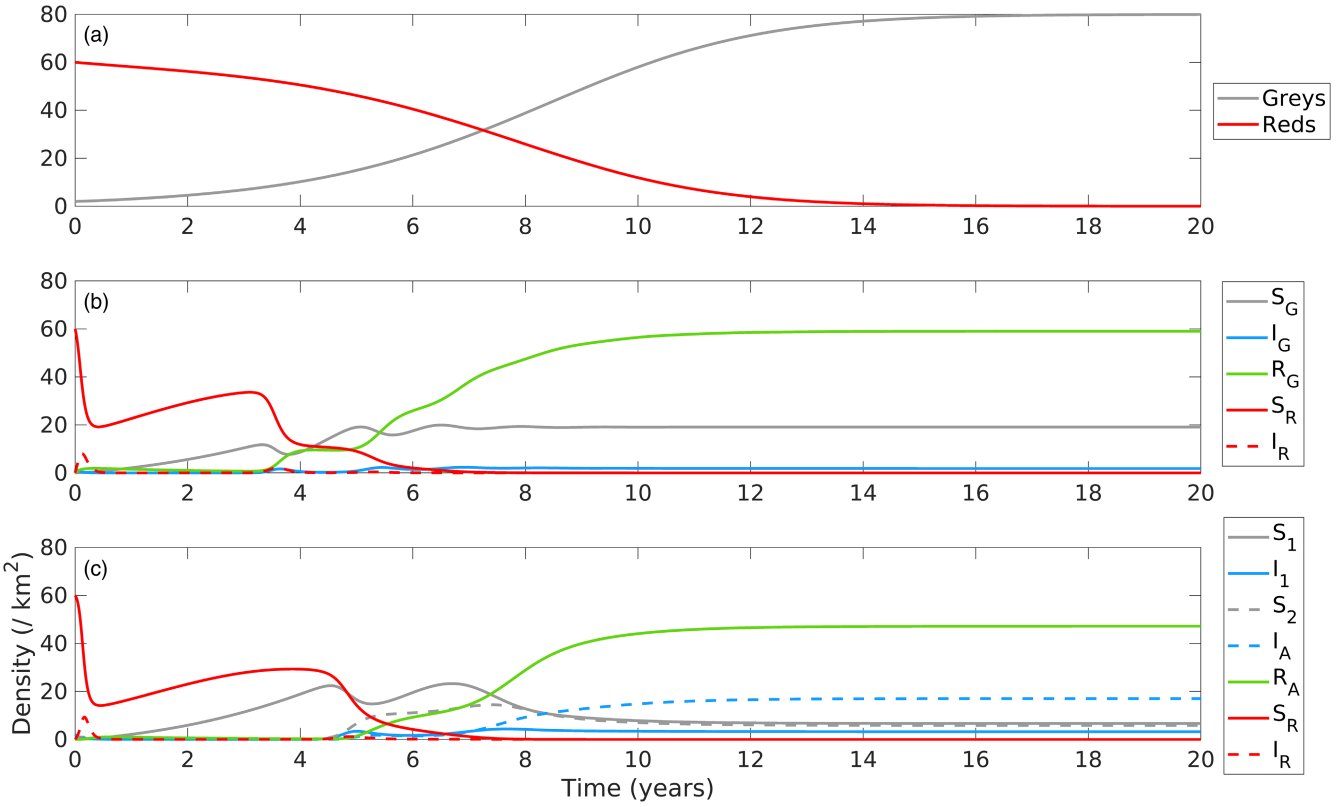

FIGURE 2 Population density against time for (a) the competition only model of Tompkins et al. (2003), (b) the competition and disease model of Tompkins et al. (2003); (c) the model represented by Equations (6) where $\gamma = 8.7$/year, $\sigma = 0.62$/year, $\beta_G = 0.22$km^2/year and $\beta_R = 0.82$km^2/year as determined in Section 3.3. Other parameters are as described in Tompkins et al. (2003). In (a) and (b) the populations classes shown are susceptible, S_G, infected, I_G, and recovered/immune, R_G, grey squirrels and susceptible, S_R, and infected, I_R, red squirrels. In (C) the population classes are as described in Section 3.4. In (a–c) red squirrels are initially at their carrying capacity and in (a) one susceptible grey squirrel and (b, c) one infected grey squirrel is introduced.

population crash is driven by infection transmission between red squirrels (which is similar in our model and Tompkins et al., 2003). As grey squirrels increase in density, between species SQPV transmission initiates a secondary outbreak of SQPV within the remaining red squirrel population and grey squirrels competitively exclude the SQPV depressed red squirrel population more rapidly than if SQPV was absent and the red squirrel density was higher. By the time infection levels in the grey squirrel population are high the red squirrel population has largely been excluded by grey squirrels (and then the endemic level of SQPV in grey squirrels is significantly higher in Figure 2c than 2b). Therefore, while transmission of SQPV from grey to red squirrels is important, disease-mediated replacement of red squirrels is largely driven by SQPV transmission within red squirrels and the increasing impact of competition from grey squirrels.

3.5 | The role of habitat in disease-mediated invasion

To explore the impact of the modified grey and red squirrel epidemiological dynamics on red squirrel population persistence and conservation, we examine the potential for disease-mediated, spatial, invasion of grey squirrels into established red squirrel populations in different habitats. To do this, we consider a spatial, stochastic model extension of Equations (6). To generate the stochastic model, we consider an array of 1 km by 1 km connected patches within an idealised 10 km by 20 km arena. We assume that red squirrels, at their carrying capacity, occupy the left half (10 km by 10 km) of the arena and grey squirrels, at their carrying capacity, occupy the right half of the arena. We also change the carrying capacity to represent conifer or mixed, mostly broadleaf dominated habitat in different halves of the arena where for conifer, $K_R = 23$/km^2 and $K_G = 10$/km^2 (Slade et al., 2020) and for the mixed habitat $K_R = 60$/km^2 and $K_G = 80$/km^2 (as in Section 3.4). When we consider the role of SQPV, we additionally assume that two grey squirrels, in alternate grid squares, at the right hand edge of the arena, are infected with SQPV. The rates in the deterministic model (Equation 6) are converted into probabilities of events that account for changes in individual grid-square level abundance from Renshaw (1993), and dispersal can occur between patches (see Supporting Information Section S.4 for full details of the stochastic model). The stochastic model is run for 20 realisations, allows for local population and disease extinction, and has been used to model red and grey squirrel and SQPV dynamics in different habitats (Jones et al., 2017; Slade et al., 2020; White et al., 2016). The stochastic framework will allow an assessment of

how the updated red and grey squirrel epidemiological dynamics impact current understanding of disease-mediated replacement of red by grey squirrels in the UK.

When we assume that the habitat represents conifer (a low-density habitat for both species, but especially for grey squirrels) across the entire spatial arena, we see that there is little discernible difference between model runs with and without SQPV (Figure 3A(i,ii)). Here, SQPV cannot be supported in the low-density grey or red populations and fades out within the first year of all simulations (see Supporting Information Figure S.4A(i,ii)). Since red squirrels are assumed to have a sufficiently higher carrying capacity in conifer compared to grey squirrels, Slade et al. (2020), red squirrels can out-compete and replace grey

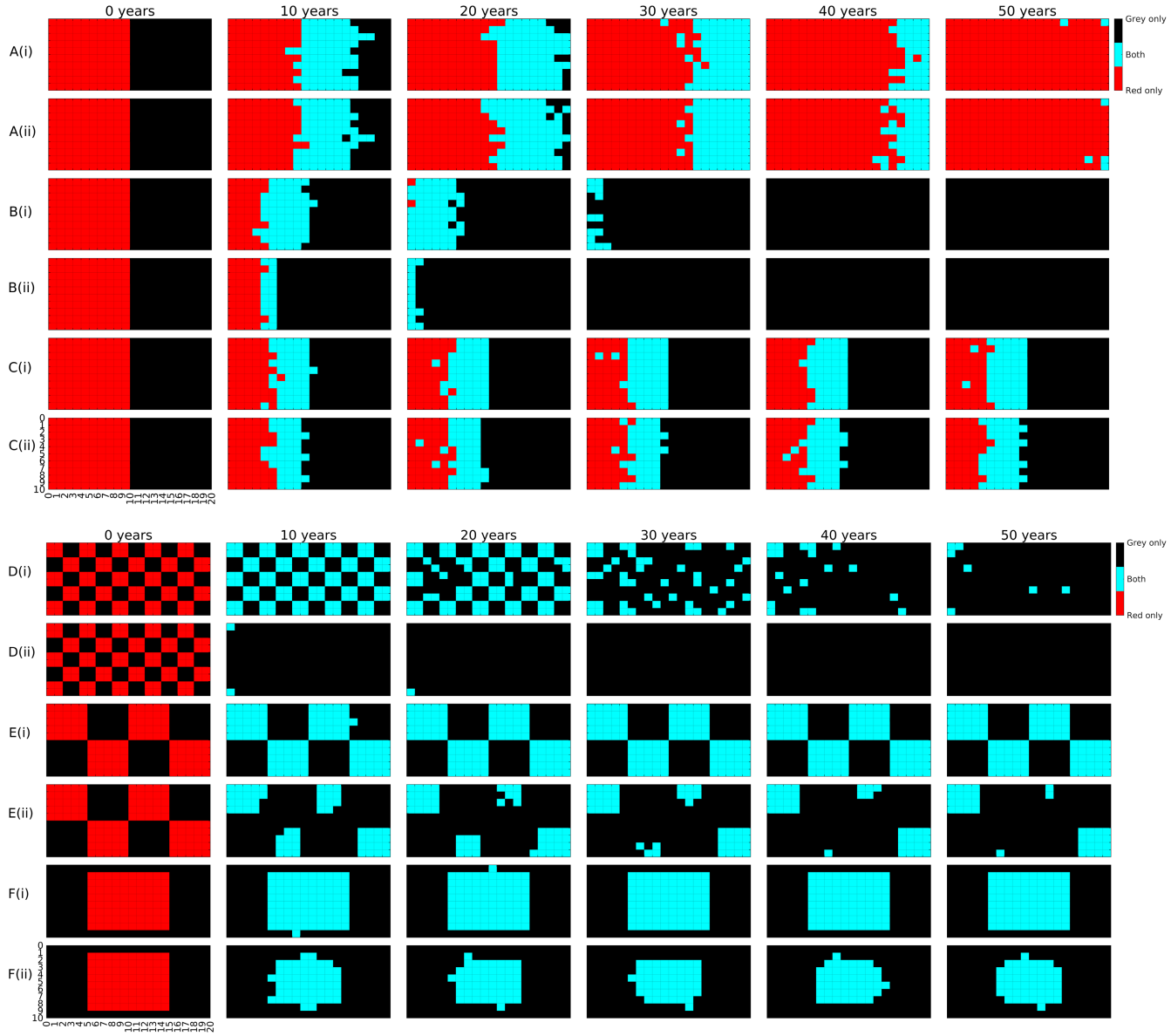

FIGURE 3 Results from the spatial, stochastic model (see Supporting Information Section S.4) indicating grid squares that are occupied by red squirrels (red), grey squirrels (black) or both red and grey squirrels (blue). Occupancy requires a presence of 2 individuals or 10% of the carrying capacity, whichever is larger, in more than 50% of the model realisations (out of a total of 20 realisations). In (A(i)) and (A(ii)) the habitat is conifer for the entire spatial arena, in (B(i)) and (B(ii)) the habitat is mixed, mostly broadleaf trees for the entire arena, and in (C(i)) and (C(ii)) the left half (10×10 grid squares) is conifer and the right half mixed trees. In (A(i)), (B(i)) and (C(i)), SQPV is absent. In (A(ii)), (B(ii)) and (C(ii)) SQPV is present and initiated by introducing 2 infected grey squirrels to every other grid square on the farthest right of the arena. In (D–F), there is a checkerboard habitat distribution, where in (D(i)) and (D(ii)), the habitat is alternating 2×2 grid squares of conifer and mixed trees, in (E(i)) and (E(ii)), the habitat is alternating 5×5 grids squares of conifer and mixed trees, in (F(i)) and (F(ii)), the centre 10×10 square is conifer and it is surrounded by mixed habitat. In graphs (D–F), the initial conditions are red squirrels at their carrying capacity in conifer habitat and greys at their carrying capacity in mixed habitat. In (D(i)), (E(i)) and (F(i)), SQPV is absent. In (D(ii)), (E(ii)) and (F(ii)), SQPV is present and initiated by introducing two infected grey squirrels to every other grid square on the farthest right of the arena in grey squirrel occupied grid cells.

squirrels in conifer dominated habitats (this requires $K_G < c_R K_R$). Long-term red squirrel persistence in the presence of nearby grey squirrels has been observed in the UK in large conifer plantations managed for forestry (Bryce et al., 2002; Slade et al., 2020).

When we assume the habitat represents mixed, mostly broadleaf dominated trees across the entire arena the replacement of red by grey squirrels is more rapid in the presence of SQPV (Figure 3B(i,ii)). Here, SQPV outbreaks in red only populations occur in advance of the replacement of grey squirrels (Supporting Information Figure S.4B(i,ii)), this reduces red squirrel density and allows grey squirrels to replace red squirrels more rapidly.

When the left half of the arena represents conifer habitat (occupied by red squirrels) and the right half of the arena represents mixed, mostly broadleaf habitat (occupied by grey squirrels) there is a slight expansion of the region of grey squirrel occupancy at the boundary between the conifer and mixed habitats when SQPV is included (Figure 3C(i,ii)). Grey squirrels can persist in conifer, at low density at the interface between the mixed and conifer habitats, but do not replace red squirrels and do not expand their distribution throughout the conifer habitat. SQPV persists in grey squirrel populations in mixed habitat and this leads to outbreaks in red squirrel populations at the interface between conifer and mixed habitats (Supporting Information Figure S.4C(i,ii)) where both squirrel species persist and this accounts for the slight expansion of the grey squirrel distribution when SQPV is present. SQPV does not spread through the red squirrel only populations in conifer habitat (Supporting Information Figure S.4B(i,ii)) since K_R is below the threshold density that supports the infection.

To further understand the role of habitat composition on red squirrel conservation, we consider idealised, 'checkerboard' habitat composition of 2 km, 5 km or 10 km alternating squares of conifer and mixed habitat (Figure 3D–F). When the habitat is composed of 2 km squares, the replacement of red squirrels by greys occurs in all patches (Figure 3D(i,ii)) but the replacement is more rapid when SQPV is present. When the habitat is composed of 5 km squares red squirrels can persist in the all conifer blocks in the absence of SQPV. When SQPV is present red squirrels can only persist in the conifer corner blocks that have less neighbouring, grey squirrel occupied, mixed habitat (Figure 3E(i,ii)). When that habitat is initially composed of a red squirrel occupied 10 km conifer block surrounded by grey squirrel occupied mixed habitat then red squirrels can survive both in the absence and presence of SQPV but the region of red squirrel occupancy and the red squirrel density is reduced when SQPV is present (Figure 3F(i,ii)). For the equivalent plots of Figure 3(D–F) showing red and grey squirrel density see Supporting Information Figures S.5 and S.6 respectively.

4 | DISCUSSION

The interaction between SQPV and red and grey squirrels in the UK is an important example of where a shared pathogen has been critical in the invasion and replacement of a native population. In this system, mathematical models were important in highlighting the role of the pathogen in causing disease-mediated replacement of native red squirrels and prompted a change in conservation strategy to try to prevent SQPV spread (White et al., 2016). Recently, more field data has become available for this system and this provides the opportunity to gain a better understanding of the fundamental disease processes and their implications for conservation. We used a suite of epidemiological models to infer the fundamental processes and mechanisms that underpin the SQPV infection interactions in red and grey squirrels. Previous models of grey squirrel epidemiology (Tompkins et al., 2003; White et al., 2014, 2016) assumed life-long immunity following infection which contradicts the recent field data that shows widespread reinfection of grey squirrels with SQPV (Chantrey et al., 2019). By comparing the results of a series of different model frameworks and comparing them to data, we were able to determine the likely disease processes in the grey squirrel, SQPV system. Furthermore, we examine the implications of the new data informed model for the conservation of the endangered red squirrel in the UK.

Our first key finding is that a model of partial or waning immunity and reinfection can represent the epidemiological dynamics of SQPV in grey squirrels. The field data of Chantrey et al. (2019) show that once grey squirrels have a positive antibody response to SQPV, it is permanent, but individuals can still be infected, leading to viraemic seropositive individuals. The laboratory data of Fiegna (2012) showed that individuals become seropositive after a second challenge and we see this pattern in the field data (Chantrey et al., 2019). We found that models of partial or waning immunity fit the data when the antibody response occurs at the second infection. A model of recrudescence, where recovered individuals can revert to the infectious state without contact with an infectious individual that had been suggested to explain the data (Chantrey et al., 2019; Roberts & Heesterbeek, 2021) fails to fit the data and lacks empirical support for pox viruses (Chantrey et al., 2019). Our modelling has therefore given us the parsimonious inference that the epidemiology of SQPV in grey squirrels can be understood simply through partial or waning immunity. Importantly, however seropositivity is a much more complex marker than is usually assumed, since it takes more than one infection challenge to become seropositive which then lasts permanently (or at least longer than between bouts of reinfection). Since protection is partial or waning, however, our model requires that there are viraemic seropositive individuals, in agreement with the field data of Chantrey et al. (2019). Taken together this emphasises that simple inference from seropositivity data and the assumption of SIR epidemiological dynamics may be misleading in wildlife infectious disease. We typically have very little direct data on immunological interactions in wildlife disease, but our approach shows how we can use models to infer these processes from longitudinal field data.

We use data for a SQPV outbreak in a red squirrel stronghold at Formby, Merseyside, to determine an independent estimate for the rate of infection transmission between red squirrels. Our second key result is that we estimate that SQPV transmission between

red squirrels is nearly 4x higher than transmission between grey squirrels. It is important to note the that the transmission rate for red squirrels assumed in Tompkins et al. (2003) is similar to the one we find here, but that our estimate for grey squirrel transmission is lower. Nevertheless, our results are similar to Tompkins et al. (2003), in particular, the rate of replacement of red squirrels by greys is greatly increased when SQPV is present compared to when it is absent. Our model assumes higher infectivity of red squirrels since there is evidence that open wounds are more likely to occur in red squirrels than greys, and these lesions may be a key mechanism for shedding the virus and thus spreading infection (Fiegna, 2012). Our findings are similar if we assume red squirrels have higher susceptibility to SQPV or if they have both higher infectivity and susceptibility. In their original model, Tompkins et al. (2003) assumed that SQPV transmission both within and between red and grey squirrels was the same. They made this assumption as the parsimonious one, in the absence of data, and carried out a sensitivity analysis to see the impact of differential transmission rates. If we make the assumption that red squirrel infection transmission is the same as grey squirrel transmission for our models that can represent the field data, then SQPV has little impact on the replacement of red squirrels by greys with the basic reproductive ratio for red squirrels less than one. Field studies suggest that SQPV accelerates the replacement of red squirrels by greys (Chantrey et al., 2014; Rushton et al., 2006), and a new insight from our models is that this occurs due to higher transmission rates of infection for red squirrels.

A key feature of the field data, and our new models that can represent the data, is that the level of infection in grey squirrels is high (25%) compared to previous expectations (2.5% in the SIR model framework of Tompkins et al., 2003). This could have important implications for the disease-mediated impact of grey squirrels on red squirrels. It was therefore important that we examined the interaction under the new model assumptions of reinfection and partial immunity to SQPV in grey squirrels and higher transmission in red squirrels. The key inferences from the simpler models on the spread and impact of SQPV carrying grey squirrels on red squirrel populations still hold. In particular, we expect SQPV to spread rapidly through connected, medium to high density, populations of grey squirrels (Tompkins et al., 2003; White et al., 2016) as was observed in England, Wales and Southern Scotland in the late 20th and early 21st century (Sainsbury et al., 2000; White et al., 2016). Endemic SQPV in grey squirrels can lead to outbreaks of infection, causing high population mortality, in adjacent red squirrel populations (Chantrey et al., 2014; Shuttleworth et al., 2022; White et al., 2014, 2015) but SQPV is not supported in low density red or grey squirrel population, and in particular SQPV is still not predicted to spread through red squirrel only populations (Macpherson et al., 2016; Slade et al., 2020). SQPV may not spread through red squirrel populations as their density may be below a threshold that supports the infection (Slade et al., 2020) or due to stochastic fade out of infection following an epidemic outbreak which has been shown to be a feature of highly virulent pathogens like SQPV in red squirrels (Chantrey et al., 2014; Macpherson et al., 2016; White et al., 2014).

Our results have implications for red squirrel conservation. In red squirrel strongholds (isolated and protected red squirrel populations in England, Wales and Southern Scotland), red squirrels persist under a constant threat from grey squirrel invasion. In red squirrel strongholds that would support medium to high-density populations of red or grey squirrels, conservation may require targeted grey squirrel control in a buffer zone around the stronghold to reduce the density, dispersal, and SQPV prevalence, of the neighbouring grey squirrel populations (Chantrey et al., 2014). Even with grey squirrel control, periodic outbreaks of SQPV are likely to occur at the interface of red and grey squirrels and may result in significant red squirrel population decline (Chantrey et al., 2014; White et al., 2014). It is important to note that SQPV is just one of the factors that may lead to red squirrel replacement by grey squirrels. Even in the absence of SQPV greys can replace reds in many mixed habitat locations (as was the case in Southern Scotland prior to the arrival of SQPV at the England/Scotland border in 2005; White et al., 2016, and in Italy where the virus is absent; Martinoli et al., 2010; Wauters et al., 2023). Typically, however, the replacement has been slower in the absence of the virus.

Red squirrel populations in Northern and Western Scotland mainly persist in isolation from grey squirrels. Much of the habitat is dominated by large conifer plantations where red squirrels may have a competitive advantage over greys if they were to arrive (Slade et al., 2020). Furthermore, SQPV would be unlikely to persist as red squirrel density is low (Slade et al., 2020). Red squirrels may also persist in natural strongholds in Southern Scotland and Northern England, in large conifer plantations (Slade et al., 2021). Grey squirrels may persist in mixed habitat adjacent to the plantations, but do not out-compete red squirrels in the poor quality, conifer dominated habitat. SQPV may have an impact at the interface between red and grey squirrel populations but would fade-out and not spread extensively through red squirrel populations (Slade et al., 2020; White et al., 2015). Our new work supports these findings and also suggest that the area of favourable, conifer dominated habitat for red squirrels is critical. This echoes observations from the field in the north of England as well as Scotland. Wauters et al. (2000) examined red and grey squirrel interactions in Hamsterley Forest, north-east England, where the core of the forest was composed of high-quality deciduous habitat and the higher elevations composed of conifer forest (spruce and pine). Despite being a conifer dominated forest, the patchwork of adjoining deciduous blocks led to continuous immigration of 'surplus' grey squirrels from the deciduous to the conifer habitat. The resultant competition in the conifer blocks led to a significant reduction in juvenile red squirrels. Red squirrel adults seemed unaffected by the competition and the main competitive impact was on red squirrel juveniles. This suggests differential competitive abilities of different age groups and represents an as yet unexplored aspect in terms of field studies and model simulations. Similarly, Bryce et al. (2002) reported that red and grey squirrels had co-existed for up to 30 years in and around Craigvinean Forest in Scotland. Grey squirrels largely inhabited the deciduous, riparian woodland zone and red squirrels the extensive conifer forest behind.

While there was overlap between the two species, macro-habitat utilisation differed significantly with red squirrels selecting Norway spruce (*Picea abies*) and grey squirrels the riparian corridor with its mixed woodland. These field observations and our model predictions clearly indicate that a natural stronghold should therefore be large enough that dispersal and immigration of neighbouring grey squirrels, from mixed/broadleaf habitat, is limited to the boundary region and does not impact a suitably large core of red squirrel dominated habitat. Previous work has indicated that species conservation requires a critical habitat size for the survival and recovery of endangered species (Camaclang et al., 2015). Importantly, our model study highlights that shared and emergent disease can also play key role in determining critical habitat size. In terms of red squirrel conservation, the area of suitable habitat needed to protect red squirrels needs to be increased if they face the threat from neighbouring grey squirrels that are an endemic reservoir for SQPV.

Red squirrel populations in Northern and the Republic of Ireland and some regions of Scotland are recovering and increasing in density. The has been linked to the range expansion and population recovery of a native predator, the pine marten (Bamber et al., 2020; Sheehy et al., 2018; Sheehy & Lawton, 2014; Twining, Montgomery, et al., 2020). Pine marten have been observed to have a higher predation rate on grey squirrels compared to red squirrels and therefore provide targeted control of grey squirrels (Sheehy & Lawton, 2014; Twining, Montgomery, Price, et al., 2020). This may lead to the eradication of grey squirrels and population recovery of red squirrels (Roberts & Heesterbeek, 2021; Slade et al., 2023; Twining, Montgomery, et al., 2020). Even when the predator fails to eradicate grey squirrels it may reduce their density to levels that cannot support endemic SQPV (Slade et al., 2023). This will benefit neighbouring red squirrels by removing the chance of epidemic SQPV outbreaks and extending the regions that can support red squirrels. This highlights the benefit of natural predators in invasive species and disease control and emphasises the role of community interactions in determining the risk of disease spillover (Roberts & Heesterbeek, 2021; Slade et al., 2023; Tanner et al., 2019; Twining et al., 2022).

Our modelling shows that relatively simple mathematical models, that assume partial or waning immunity in grey squirrels, can capture the field data when most seroconversion happens at the second challenge of infection. Given that there is evidence from both experiments and the field data that seroconversion may take multiple infections, our work provides a parsimonious explanation of the epidemiology of SQPV in grey squirrels. We also show that SQPV is likely to have been an important driver of the rapid replacement of red squirrels by greys in the UK. Moreover, our work focused on the UK squirrel and SQPV case study system has general implications for understanding the impact of disease-mediated invasion of non-native species (Hatcher et al., 2006; Prenter et al., 2004; Strauss et al., 2012). It suggests that the impact will be severe when infection transmission and disease-induced mortality within the native species is pronounced. Here, transmission from the reservoir, invasive, species to the native species can lead to an epidemic outbreak in the native species that suppresses native species density and their competitive ability which can lead to replacement by the invasive species. This can occur even if the disease cannot be supported in the native species (it would fade out following the epidemic outbreak) since the invading species provides a source for re-infection. This mechanism, defined as spillover disease-mediated invasion, has been shown to drive replacement in many other systems (Strauss et al., 2012) and is often implicated in animal invasions in which the non-native species is phylogenetically similar to the native species it is replacing. However, it is not just the invading animal species that is of concern since the parasites introduced with the invasive species are themselves a non-native species (Strauss et al., 2012; Telfer & Bown, 2012). Such parasites have the potential for zoonotic spillover with risks to public and animal health (Chinchio et al., 2020) and calls for increased vigilance on the potential health risks posed by biological invasions (Hulme, 2014; Roy et al., 2017).

AUTHOR CONTRIBUTIONS

All authors conceived the ideas and designed methodology; EH and AW undertook the mathematical analysis; all authors contributed to the writing of the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data used in this study can be found in the following publications: http://hdl.handle.net/1842/17994, https://doi.org/10.1016/j.epidem.2019.100352, https://doi.org/10.1002/ece3.1216.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supporting Information Section S.1. Waning immunity models.

Supporting information Section S.2. Recrudescence model.

Supporting Information Section S.3. Interspecies infection parameters.

Supporting Information Section S.4. Stochastic model.

Supporting Information Section S.5. Additional results for the spatial, stochastic model.

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