Journal of General Virology (2003), **84**, 3337–3341 DOI 10.1099/vir.0.19464-0

Short Communication

A novel poxvirus lethal to red squirrels (*Sciurus vulgaris*)

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Received 26 June 2003
Accepted 1 September 2003

A parapoxvirus has been implicated in the decline of the red squirrel in the United Kingdom. Virus was isolated from an outbreak of lethal disease in red squirrels in the north-east of England. Experimental infection of captive-bred red squirrels confirmed that this virus was the cause of the severe skin lesions observed. Electron microscopic examination of the virus showed that it had a morphology typical of parapoxviruses whilst preliminary sequence data suggested a genomic G+C composition of approximately 66 %, again similar to that found in other parapoxviruses. However Southern hybridization analysis failed to detect three known parapoxvirus genes, two of which have been found so far only in the genus *Parapoxvirus*. Comparative sequence analysis of two other genes, conserved across the eight recognized chordopoxvirus genera, suggests that the squirrel virus represents a previously unrecognized genus of the *Chordopoxviridae*.

The prognosis for the survival of the red squirrel (*Sciurus vulgaris*) in the United Kingdom is poor. Numbers have been in decline for the last 150 years (Lloyd, 1983; Gurnell & Pepper, 1993), with extinction in mainland England possible within the next 20 years (Kenward & Holm, 1989; Gurnell & Pepper, 1993). Competition between red and grey squirrels for habitat and food undoubtedly plays a role in replacement of the red species by the grey squirrel (Skelcher, 1997). However several authors have attributed the extinction of local populations of red squirrels to the presence of an epidemic disease that causes high mortality (Sainsbury *et al.*, 1997, 2000; Rushton *et al.*, 2000; Tompkins *et al.*, 2003). The disease is characterized by ulcerated and haemorrhagic scabs affecting the skin around the eyes, nose and lips which later spread to the ventral thorax, inguinal area and feet (Edwards, 1962; Sainsbury & Gurnell, 1995). The causative agent was identified tentatively by electron microscopy as a parapoxvirus (SPPV) (Scott *et al.*, 1981). The origin of the virus is not clear, but no clinical disease was recorded until the introduction of grey squirrels to the UK (Sainsbury & Gurnell, 1995). A serological survey of red and grey squirrels from across the UK revealed that 61 % of 223 apparently healthy grey squirrels had antibodies to SPPV, whereas only 3·2 % of 140 red squirrels were seropositive (Sainsbury *et al.*, 2000). All the seropositive red squirrels were found dead or dying with symptoms typical of the poxvirus disease. There has only been one confirmed report of SPPV-induced disease in a wild grey squirrel (Duff *et al.*, 1996), and generally grey squirrels are thought to be unaffected clinically. However, the high seroprevalence of antibodies to the virus and low incidence of disease in grey squirrels suggest that they may be a reservoir for SPPV, which is then transmitted to red squirrels causing lethal disease.

Experimental infection with a mixture of isolates from various outbreaks of scab disease confirmed that SPPV is the agent responsible for the disease observed in red squirrels. In contrast, infection of grey squirrels failed to induce disease although all developed antibodies to the virus (Tompkins *et al.*, 2002). We have taken a single isolate of the virus from an outbreak of disease in northern England and shown that it alone causes a deleterious disease in red squirrels. Sequencing and subsequent phylogenetic analysis of genes from this virus casts doubt over its relationship to the parapoxviruses.

Virus was isolated from a red squirrel (see supplementary data at JGV Online: http://vir.sgmjournals.org) with typical SPPV-induced haemorrhagic erythematous dermatitis about the face and on the feet and was visualized by negative-staining transmission electron microscopy. The SPPV particles were generally ovoid with approximate dimensions of 275 × 175 nm, similar to the parapoxviruses. The regular basket-weave surface morphology characteristic of the parapoxviruses was also observed (Fig. 1A). However, the angle at which the SPPV basket-weave ridges cross each other

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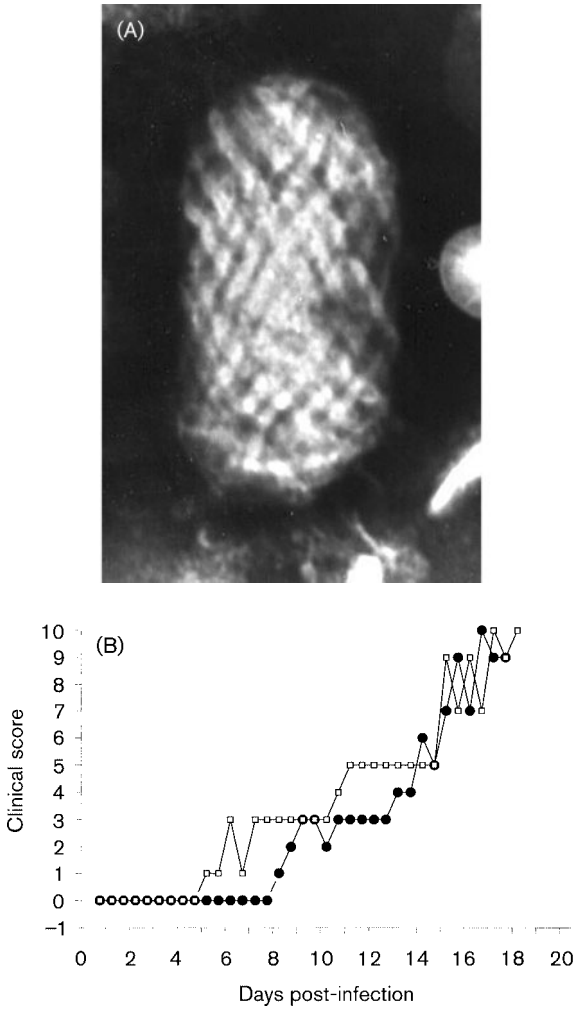

Fig. 1. (A) Virus from the scabs on a squirrel (1296/99) found dead in Northumberland, England, exhibiting typical poxvirus-like lesions on the head, hands and feet (see supplementary data at JGV Online: http://vir.sgmjournals.org), was visualized by negative-staining transmission electron microscopy. (B) Two captive-bred red squirrels were infected with virus isolated directly from scab material taken from squirrel 1296/99. Scabs were homogenized in PBS and after clarification at 2000 *g* for 30 min at 4 °C, the virus was purified by centrifugation at 71 000 *g* for 30 min at 4 °C through a sucrose cushion (36 %, w/w, in PBS). The virus pellet was re-suspended in PBS, the concentration estimated by electron microscopy and adjusted to approximately 10^5 virus particles ml^{-1}. The squirrels were infected with the virus suspension and their health was monitored every 12 h according to the clinical scoring method of Tompkins *et al.* (2002). Animals exhibiting a clinical score of 8 or more on three consecutive occasions were removed from the experiment.

appears to differ from that of other parapoxviruses, supporting the previous observations made by Scott *et al.* (1981).

Confirmation that this virus could induce scab disease was obtained by experimental infection of two captive-bred red squirrels. Virus was isolated directly from scab material and its concentration was estimated by electron microscopy and adjusted to approximately 10^5 virus particles ml^{-1} in PBS. Squirrels were challenged by topical application of 0·2 ml virus suspension to a scarification site on the right thigh and also by subcutaneous injection of 0·2 ml into the right thigh. The progression of disease in each animal was assessed every 12 h using the clinical scoring system described by Tompkins *et al.* (2002). Individuals scoring eight or more on three consecutive occasions were removed from the experiment, as it was predetermined that this severity of disease would cause mortality in the wild. Both squirrels succumbed to disease within 10 days. Severe secondary lesions appeared within 2 weeks and the animals exhibited lethargy and poor condition, appetite loss by up to 100 % and reduction in body weight by up to 12 %. The progression of disease in both individuals (Fig. 1B) was not significantly different from that reported previously (ANOVA $F_{1,3}=0{\cdot}06$, $P=0{\cdot}83$), with animals being removed from the experiment at days 17·5 and 18·0, compared to days 15·0, 17·0 and 18·5 in the previous study with the mixture of viruses. The overall pathological symptoms were consistent with those reported previously in wild red squirrels (Edwards, 1962; Sainsbury & Gurnell, 1995), and confirmed that the isolated virus was virulent.

SPPV has been formally classified as a *Parapoxvirus* (van Regenmortel *et al.*, 2000), although Sands *et al.* (1984) concluded that SPPV and *Orf virus* (ORFV) were antigenically distinct. Furthermore, in a study of 27 monoclonal antibodies (mAbs) raised against ORFV, only two were found to cross-react with SPPV (Housawi *et al.*, 1998). This contrasts with 17 mAbs that cross-reacted with both *Pseudocowpox virus* (PCPV) and *Bovine papular stomatitis virus* (BPSV) and six that cross-reacted with Sealpox virus, suggesting that SPPV is more divergent from ORFV than from these other parapoxviruses. To study the relationship between SPPV and the other parapoxviruses we attempted to identify genes within the SPPV genome that were related to known ORFV genes and in particular to those associated with modulation of the host immune response and/or virulence. Five cosmid clones that span the SPPV genome were screened for the presence of sequence related to the ORFV *GIF* gene (encoding a protein capable of binding interleukin-2 and granulocyte–macrophage colony-stimulating factor; Deane *et al.*, 2000), the *VEGF-E* gene (encoding a protein related to mammalian vascular endothelial growth factor; Lyttle *et al.*, 1994), the *IL-10* gene (encoding a protein closely related to mammalian interleukin-10; Fleming *et al.*, 1997) and the orthologue of the *H5R* gene of *Vaccinia virus* (VACV) (encoding a late transcription factor, VLTF-4; Kovacs & Moss, 1996). Two of these genes, *VEGF-E* and *GIF*, have been found so far only in parapoxviruses whilst the IL-10 gene has also been found in the capripoxvirus *Lumpy skin disease virus* (LSDV) (Tulman *et al.*, 2001). Orthologues of *H5R* are found in each of the chordopoxvirus genera. No specific hybridization between the ORFV probes and

SPPV DNA was detected with the exception of that corresponding to the *H5R* gene (results not shown). On the basis of this it was hypothesized that SPPV does not encode orthologues of any of the ORFV *GIF*, *VEGF* or *IL-10* genes or that the corresponding SPPV sequences have diverged sufficiently so as to prevent cross-hybridization. Comparative sequence analysis of 22 ORFV strains and isolates demonstrated that the *VEGF-E* gene varies considerably even within a single species of virus (Mercer *et al.*, 2002). This heterogeneity could explain our inability to identify an orthologue of *VEGF-E* in SPPV by hybridization. *VEGF-E* genes are present close to the right ITR junctions in two of the three parapoxviruses for which there is sequence data available, that is in ORFV and PCPV, but not in BPSV (Lyttle *et al.*, 1994; Rziha *et al.*, 2003; Ueda *et al.*, 2003). We sequenced approximately 1 kb of the corresponding region of the SPPV genome, but were unable to identify an orthologue of *VEGF-E*. Instead an ORF with similarity to the *003L* and *157R* genes of *Molluscum contagiosum virus* (MOCV) was found (results not shown). These genes appear to represent paralogues of each other although they do differ at their 3′ ends (Senkevich *et al.*, 1997). The SPPV ORF (GenBank accession no. AY312570) is approximately 49 % identical to the MOCV sequences, with the predicted amino acid sequences sharing approximately 28 % identity.

The hybridization and sequence data taken together suggest that SPPV probably does not possess an orthologue of ORFV *VEGF-E*. This was unexpected because the appearance of SPPV-induced lesions suggests an underlying vascularization similar to that of ORFV-induced lesions. The vascularization appears to be a function of VEGF-E since knockout viruses lacking this protein produced lesions that had lower blood vessel formation in the dermis than lesions induced with wild-type virus and as a consequence were considerably less erythematous and florid (Savory *et al.*, 2000).

Since we were unable to identify known parapoxvirus genes associated with immunomodulation or virulence within the SPPV genome we sought alternative genes with which to explore the relationship between SPPV and other parapoxviruses. Phylogenetic analysis with the partial sequence of the major outer envelope protein (orthologue of VACV F13L) has been used to infer speciation within the genus *Parapoxvirus* (Becher *et al.*, 2002). Previous analysis was based on the alignment of amino acids 137 to 320 (numbering with respect to the ORFV protein) because the data produced for the majority of parapoxviruses are derived from PCR products using primers considered to be specific for all parapoxviruses (Inoshima *et al.*, 2000). Attempts to obtain a PCR product from SPPV DNA using these primers failed. Nevertheless we identified, by DNA hybridization with the ORFV gene, a plasmid containing the corresponding SPPV sequence. Sequence analysis revealed the presence of an ORF (GenBank accession no. AY312569) corresponding to amino acids 129 to 344 of the ORFV protein. Pairwise alignment of the predicted SPPV amino acid sequence with that of the four recognized species of *Parapoxvirus*, ORFV, PCPV, BPSV and *Parapoxvirus of red deer in New Zealand* (PVNZ), and the tentative member of the genus, Sealpox virus, indicated that the SPPV protein shared approximately 49 % identity to each. This was less than with VACV F13L (57 % identity). We aligned our sequence with all known chordopoxvirus orthologues of the F13L protein, representing all eight chordopoxvirus genera, and constructed a phylogenetic tree based on a maximum-likelihood algorithm (Fig. 2A). The analysis grouped ORFV, BPSV, PCPV and PVNZ together with Sealpox virus, but the SPPV sequence was placed on a separate branch of the tree with 82 % support from the bootstrap resampling. Indeed, the SPPV sequence was placed on a branch of its own and did not partition with any of the other poxvirus genera, suggesting that SPPV represents a separate genus of the *Poxviridae*.

To gather support for this theory we performed a similar phylogenetic analysis on a second SPPV gene. The orthologue of the VACV *E4L* gene (encoding the 30 kDa RNA polymerase subunit; Ahn *et al.*, 1990) was identified by random sequencing of plasmid clones derived from the SPPV genome. It was predicted to encode a protein of 297 amino acids, 38 amino acids longer than the VACV protein. Likewise, it is predicted to be 96, 75, 75, 115, 108 and 104 amino acids longer than the corresponding proteins in the *Capripox*, *Leporipox*, *Suipox*, *Avipox*, *Yatapox* and *Parapox* genera, respectively. In contrast, the MOCV orthologue is predicted to be 147 amino acids longer than the SPPV sequence. Due to the variation in length between the different orthologues an initial alignment of the 17 available sequences, representing the eight different chordopoxvirus genera (and including the SPPV sequence), was performed. This indicated that there was a core of approximately 180 amino acids that were well conserved across all the genera. A phylogenetic tree based on an alignment of these 'core' amino acids was constructed. Bootstrap resampling, based on 100 data sets, was used to examine the statistical significance of the observed clades. Once again the SPPV sequence appeared on a branch of its own in the phylogenetic tree (Fig. 2B), separated from the ORFV sequence (the only parapoxvirus sequence available) with 95 % support from the bootstrapping exercise, and 64 % from the other poxvirus genera. This particular analysis, however, failed to separate the orthopoxviruses from the *Leporipox*, *Suipox*, *Capripox* and *Yatapox* virus genera with any strong bootstrapping support. Therefore, although this phylogenetic tree supports the general observation that SPPV does not readily partition with the parapoxvirus ORFV, the exact relationship between it and the other chordopoxvirus genera is less well defined.

Despite similarities in the base composition, virion morphology and in the pathological symptoms of the disease caused by SPPV when compared to the other parapoxviruses, the phylogenetic analysis presented here provides no support to classify SPPV in the genus

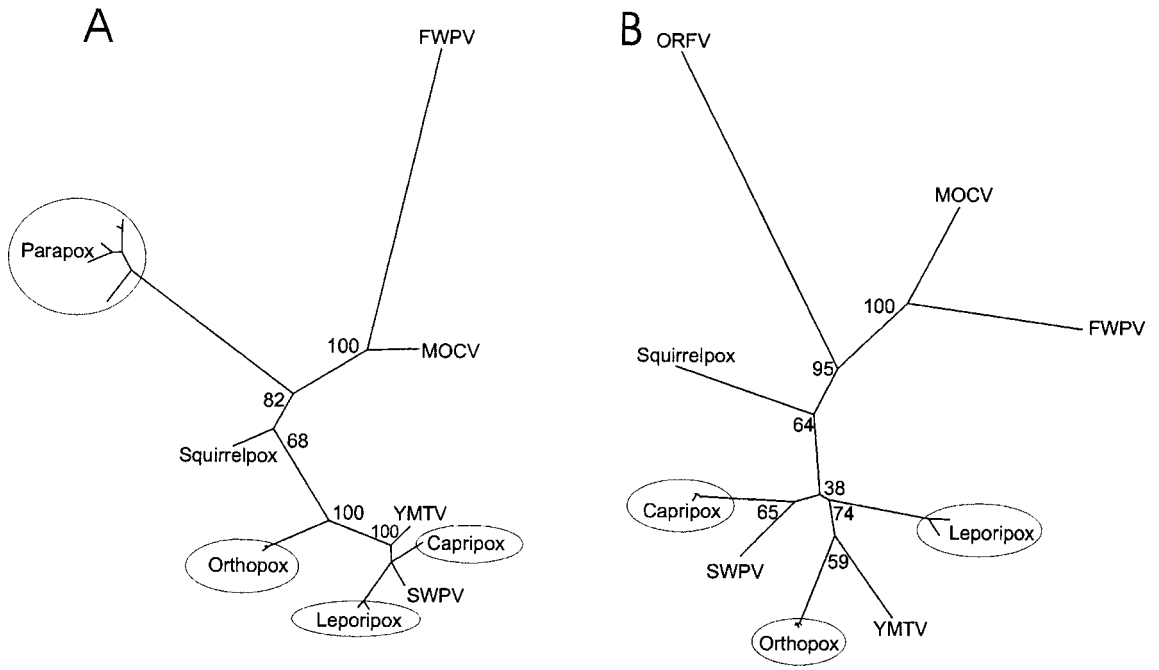

Fig. 2. Phylogenetic analysis was performed on orthologous amino acid sequences aligned using the ClustalW algorithm. Maximum-likelihood trees were generated using the PHYLIP PROML program. The model of amino acid substitution used was that of Jones/Taylor/Thornton (Jones *et al.*, 1992), and a mixed model of rate heterogeneity, with four gamma rates and one invariant rate, was used. The input order of the aligned sequences was jumbled 10 times and the global rearrangement option was selected. The gamma distribution parameter alpha and the fraction of invariable sites were calculated using the TREE-PUZZLE program (Schmidt *et al.*, 2002). The trees were evaluated using 100 bootstrap replicates (Felsenstein, 1985) and a consensus tree produced using the CONSENSE program. Trees were visualized using the graphical software TREEVIEW version 1.6.6 (Page, 1996). Branch lengths are proportionate to genetic distances and numbers indicate the percentage of bootstrap replicates that support each of the internal branches. Where several members of a genus were analysed and grouped together the name of the genus is indicated. When only one member of a genus was used in the analysis the species is indicated. (A) Phylogenetic analysis of 20 orthologues of the VACV major outer envelope protein (encoded by VACV *F13L*). The tree is based on an alignment of amino acids 137 to 313 (numbering with respect to the VACV-COP protein). The accession numbers for each of the sequences used in this analysis are; SPPV (AY312569), ORFV (Q84145), BPSV (Q91UK2), PCPV (Q91UK3), PVNZ (Q91UK1), Sealpox virus (Q8JTI4), VACV (P20638), *Variola virus* (VARV; Q85369), *Cowpox virus* (CPXV; Q8QN05), *Camelpox virus* (CMLV; Q8V2W9), *Ectromelia virus* (ECTV; Q8JLG8), *Swinepox virus* (SWPV; Q9IW61), LSDV (Q9QCQ1), *Sheeppox virus* (Q9QCQ2), *Yaba monkey tumour virus* (YMTV; Q9DHT5), MOCV (Q98189), *Fowlpox virus* (FWPV; P36316), *Rabbit fibroma virus* [SFV ('Shope fibroma virus'); Q9Q949], *Myxoma virus* (MYXV; Q83604) and *Monkeypox virus* (MPXV; Q8V535). (B) Phylogenetic analysis of 17 orthologues of the VACV RNA polymerase 30 kDa subunit (encoded by VACV *E4L*). The tree is based on the alignment of amino acids 23 to 199 (numbering with respect to the VACV-COP sequence). The accession numbers for each of the sequences used in this analysis are; SPPV (AY310357), ORFV (AY299390), *Sheeppox virus* (Q84152), SWPV (Q8V3R1), MYXV (Q9Q8R6), VACV (P21082), YMTV (Q9DHS7), LSDV (Q8JU11), *Goatpox virus* (GTPV; Q11319), CMLV (Q8V2W1), MPXV (Q8V527), ECTV (AAM92348), CPXV (Q8QMZ7), VARV (Q85373), SFV (Q9Q941), FWPV (Q9J5C0) and MOCV (Q98202).

Parapoxvirus. However, a much more extensive study of the SPPV genome and the genes it contains will be required before the virus can be classified with certainty. This classification will be important when considering future epidemiological and ecological studies.

Whilst preparing this manuscript we noted that the standard abbreviation given to the *Squirrelpox virus* (SPPV) is the same as has been given to *Sheeppox virus* (van Regenmortel *et al.*, 2000). We would like to suggest that the alternative abbreviation SQPV be adopted for *Squirrelpox virus*.

ACKNOWLEDGEMENTS

This work was funded by The Sir James Knott Trust, NERC and SEERAD. Thanks go to VLA Lasswade for electron microscopy and to Frank Wright for advice on phylogenetic analysis. We also thank John Gurnell for his role in the red squirrel surveillance programme by which diseased animals were brought to our notice.

REFERENCES

Ahn, B. Y., Gershon, P. D., Jones, E. V. & Moss, B. (1990). Identification of rpo30, a vaccinia virus RNA polymerase gene with structural similarity to a eucaryotic transcription elongation factor. *Mol Cell Biol* **10**, 5433–5441.

Becher, P., Konig, M., Muller, G., Siebert, U. & Thiel, H. J. (2002). Characterization of sealpox virus, a separate member of the parapoxviruses. *Arch Virol* **147**, 1133–1140.

Deane, D., McInnes, C. J., Percival, A. & 7 other authors (2000). Orf virus encodes a novel secreted protein inhibitor of granulocyte-macrophage colony-stimulating factor and interleukin-2. *J Virol* **74**, 1313–1320.

Duff, J. P., Scott, A. & Keymer, I. F. (1996). Parapox virus infection of the grey squirrel. *Vet Rec* **138**, 527.

Edwards, F. B. (1962). Red squirrel disease. *Vet Rec* **74**, 739–741.

Felsenstein, J. (1985). Confidence intervals on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fleming, S. B., McCaughan, C. A., Andrews, A. E., Nash, A. D. & Mercer, A. A. (1997). A homolog of interleukin-10 is encoded by the poxvirus orf virus. *J Virol* **71**, 4857–4861.

Gurnell, J. & Pepper, H. (1993). A critical look at conserving the British red squirrel *Sciurus vulgaris*. *Mammal Rev* **23**, 127–137.

Housawi, F. M. T., Roberts, G. M., Gilray, J. A., Pow, I., Reid, H. W., Nettleton, P. F., Sumption, K. J., Hibma, M. H. & Mercer, A. A. (1998). The reactivity of monoclonal antibodies against orf virus with other parapoxviruses and the identification of a 39 kDa immunodominant protein. *Arch Virol* **143**, 2289–2303.

Inoshima, Y., Morooka, A. & Sentsui, H. (2000). Detection and diagnosis of parapoxvirus by the polymerase chain reaction. *J Virol Methods* **84**, 201–208.

Jones, D. T., Taylor, W. R. & Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* **8**, 275–282.

Kenward, R. E. & Holm, J. L. (1989). What future for British red squirrels? *Biol J Linn Soc* **38**, 83–89.

Kovacs, G. R. & Moss, B. (1996). The vaccinia virus H5R gene encodes late gene transcription factor 4: purification, cloning, and overexpression. *J Virol* **70**, 6796–6802.

Lloyd, H. G. (1983). Past and present distribution of red and grey squirrels. *Mammal Rev* **13**, 69–80.

Lyttle, D. J., Fraser, K. M., Fleming, S. B., Mercer, A. A. & Robinson, A. J. (1994). Homologs of vascular endothelial growth factor are encoded by the poxvirus orf virus. *J Virol* **68**, 84–92.

Mercer, A. A., Wise, L. M., Scagliarini, A. & 7 other authors (2002). Vascular endothelial growth factors encoded by Orf virus show surprising sequence variation but have a conserved, functionally relevant structure. *J Gen Virol* **83**, 2845–2855.

Page, R. D. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.

Rushton, S. P., Lurz, P. W. W., Gurnell, J. & Fuller, R. (2000). Modelling the spatial dynamics of a parapoxvirus disease in red and grey squirrels: a possible cause of the decline of the red squirrel in the UK. *J Appl Ecol* **37**, 997–1012.

Rziha, H. J., Bauer, B., Adam, K. H., Rottgen, M., Cottone, R., Henkel, M., Dehio, C. & Buttner, M. (2003). Relatedness and heterogeneity at the near-terminal end of the genome of a parapoxvirus bovis 1 strain (B177) compared with parapoxvirus ovis (*Orf virus*). *J Gen Virol* **84**, 1111–1116.

Sainsbury, A. W. & Gurnell, J. (1995). An investigation into the health and welfare of red squirrels, *Sciurus vulgaris*, involved in reintroduction studies. *Vet Rec* **137**, 367–370.

Sainsbury, A. W., Nettleton, P. & Gurnell, J. (1997). In *The Conservation of Red Squirrels, Sciurus vulgaris* L., pp. 105–108. Edited by J. Gurnell & P. Lurz. London: People's Trust for Endangered Species.

Sainsbury, A. W., Nettleton, P., Gilray, J. & Gurnell, J. (2000). Grey squirrels have a high seroprevalence to a parapoxvirus associated with deaths in red squirrels. *Anim Conserv* **3**, 229–233.

Sands, J. J., Scott, A. C. & Harkness, J. W. (1984). Isolation in cell culture of a poxvirus from the red squirrel (*Sciurus vulgaris*). *Vet Rec* **114**, 117–118.

Savory, L. J., Stacker, S. A., Fleming, S. B., Niven, B. E. & Mercer, A. A. (2000). Viral vascular endothelial growth factor plays a critical role in orf virus infection. *J Virol* **74**, 10699–10706.

Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. (2002). TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18**, 502–504.

Scott, A. C., Keymer, I. F. & Labram, J. (1981). Parapoxvirus infection of the red squirrel (*Sciurus vulgaris*). *Vet Rec* **109**, 202.

Senkevich, T. G., Koonin, E. V., Bugert, J. J., Darai, G. & Moss, B. (1997). The genome of molluscum contagiosum virus: analysis and comparison with other poxviruses. *Virology* **233**, 19–42.

Skelcher, G. (1997). In *The Conservation of Red Squirrels, Sciurus vulgaris* L., pp. 67–78. Edited by J. Gurnell & P. Lurz. London: People's Trust for Endangered Species.

Tompkins, D. M., Sainsbury, A. W., Nettleton, P., Buxton, D. & Gurnell, J. (2002). Parapoxvirus causes a deleterious disease in red squirrels associated with UK population declines. *Proc R Soc Lond B Biol Sci* **269**, 529–533.

Tompkins, D. M., White, A. R. & Boots, M. (2003). Ecological replacement of native red squirrels by invasive greys driven by disease. *Ecol Lett* **6**, 1–8.

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Kutish, G. F. & Rock, D. L. (2001). Genome of lumpy skin disease virus. *J Virol* **75**, 7122–7130.

Ueda, N., Wise, L. M., Stacker, S. A., Fleming, S. B. & Mercer, A. A. (2003). Pseudocowpox virus encodes a homolog of vascular endothelial growth factor. *Virology* **305**, 298–309.

van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L. & 8 other editors (2000). *Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 137–157. San Diego: Academic Press.