JVI Journals.ASM.org

Comparative Analysis of the Complete Genome Sequence of the California MSW Strain of Myxoma Virus Reveals Potential Host Adaptations

Peter J. Kerr,[a] Matthew B. Rogers,[b,c] Adam Fitch,[c] Jay V. DePasse,[c] Isabella M. Cattadori,[d] Peter J. Hudson,[d] David C. Tscharke,[e] Edward C. Holmes,[f,g] Elodie Ghedin[b,c]

CSIRO Ecosystem Sciences, Canberra, Australian Capital Territory, Australia[a]; Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA[b]; Center for Vaccine Research, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA[c]; Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park, Pennsylvania, USA[d]; Research School of Biology, The Australian National University, Canberra, Australian Capital Territory, Australia[e]; Sydney Emerging Infections & Biosecurity Institute, School of Biological Sciences, and Sydney Medical School, The University of Sydney, Sydney, Australia[f]; Fogarty International Center, National Institutes of Health, Bethesda, Maryland, USA[g]

Myxomatosis is a rapidly lethal disease of European rabbits that is caused by myxoma virus (MYXV). The introduction of a South American strain of MYXV into the European rabbit population of Australia is the classic case of host-pathogen coevolution following cross-species transmission. The most virulent strains of MYXV for European rabbits are the Californian viruses, found in the Pacific states of the United States and the Baja Peninsula, Mexico. The natural host of Californian MYXV is the brush rabbit, *Sylvilagus bachmani*. We determined the complete sequence of the MSW strain of Californian MYXV and performed a comparative analysis with other MYXV genomes. The MSW genome is larger than that of the South American Lausanne (type) strain of MYXV due to an expansion of the terminal inverted repeats (TIRs) of the genome, with duplication of the *M156R*, *M154L*, *M153R*, *M152R*, and *M151R* genes and part of the *M150R* gene from the right-hand (RH) end of the genome at the left-hand (LH) TIR. Despite the extreme virulence of MSW, no novel genes were identified; five genes were disrupted by multiple indels or mutations to the ATG start codon, including two genes, *M008.1L/R* and *M152R*, with major virulence functions in European rabbits, and a sixth gene, *M000.5L/R*, was absent. The loss of these gene functions suggests that *S. bachmani* is a relatively recent host for MYXV and that duplication of virulence genes in the TIRs, gene loss, or sequence variation in other genes can compensate for the loss of *M008.1L/R* and *M152R* in infections of European rabbits.

The introduction of *Myxoma virus* (MYXV), the cause of myxomatosis, into the European rabbit (*Oryctolagus cuniculus*) population of Australia and the subsequent evolution of virus and rabbit form a classic example of host-pathogen coevolution. MYXV (family *Poxviridae*; subfamily *Chordopoxvirinae*; genus *Leporipoxvirus*) originally evolved in the Americas. Two geographically separated types of MYXV are known: the South American virus, released as a biological control into European rabbit populations in Australia and Europe, and the Californian virus, which is present in the Pacific states of the United States and the Baja Peninsula, Mexico (1, 2).

Both geographic types of MYXV naturally infect leporids of the genus *Sylvilagus*: *Sylvilagus brasiliensis* (tapeti) in South America and *Sylvilagus bachmani* (brush rabbit) in California. In its native hosts, MYXV induces an innocuous cutaneous fibroma at the site of infection; virus is passively transmitted on the mouthparts of biting arthropods, such as mosquitoes or fleas, probing through the virus-rich epidermis of the fibroma for a blood meal. However, in the European rabbit, which is not native to the Americas, both types of MYXV induce the lethal generalized disease myxomatosis, characterized by a profound suppression of innate and adaptive host immune responses (3, 4). A third leporipoxvirus, called *Rabbit fibroma virus* (RFV; also called Shope fibroma virus), is found in *Sylvilagus floridanus* (eastern cottontail) in the eastern and central states of the United States and Ontario, Canada. RFV does not cause significant disease in immunocompetent European rabbits but genetically and antigenically is sufficiently closely related to MYXV to be used as a heterologous vaccine against myxomatosis. Each virus appears to be well adapted to its natural host, based on transmission studies and the lack of serious disease (1, 5, 6), and it has been assumed that this indicates a long period of host-pathogen coevolution quite distinct from the rapid emergence and host adaptation of South American strains of MYXV in the novel European rabbit host in Australia and Europe (1, 3, 7–9).

Myxomatosis in California was first described for farmed European rabbits in the 1930s (10). Fenner and Marshall (11) characterized two isolates of Californian MYXV: MSD (San Diego 1949) and MSW (San Francisco 1950). Similar to South American strains of MYXV, these viruses were highly lethal in European rabbits, with case fatality rates of essentially 100%, but most infected rabbits died before developing the classic swollen head, ears, eyelids, and perineum of myxomatosis. In addition, high titers of virus were found in the brains of infected rabbits, and some rabbits exhibited neurological signs such as convulsions (1). Thus, the Californian viruses were considered to be neurotropic. Subsequent studies with MSW have confirmed the extreme virulence of the virus for European rabbits, both fully susceptible laboratory rabbits and Australian wild rabbits with genetic resistance

Received 12 July 2013 **Accepted** 22 August 2013
Published ahead of print 28 August 2013
Address correspondence to Elodie Ghedin, elg21@pitt.edu.

doi:10.1128/JVI.01923-13

to MYXV (12). However, in these studies, high titers of virus were not found in the brain.

The complete nucleotide sequences of the Lausanne (Lu) strain of South American MYXV and the Kazza strain of RFV have been described previously (13–15). The Lu genome consists of 161,777 bp of double-stranded DNA (dsDNA) with terminal inverted repeats (TIRs) of 11,577 bp and closed single-stranded hairpin loops at each terminus. There are 158 unique open reading frames (ORFs), 12 of which are duplicated in the TIRs. RFV has a genome of 159,857 bp carrying 151 unique genes, 12 of which are duplicated or partly duplicated in the 12,397-bp TIRs. Orthologues of all the RFV genes are present in equivalent positions in MYXV, but seven ORFs in MYXV are present only as fragments or are missing in RFV.

Both the Lu and RFV genomes encode multiple proteins involved in host range determination and suppression or evasion of host innate and adaptive immune responses (3, 4). In their natural hosts, these proteins are presumed to be essential for virus persistence at high titers and enable sufficient time for vector transmission; persistence in RFV can be more than 9 months, allowing overwintering in the absence of vectors. However, in European rabbits, MYXV completely overwhelms the host response. Gene knockout studies of Lu have implicated at least 21 MYXV genes in virulence for laboratory rabbits, and another 20 genes have potential or confirmed immune evasion or host range functions, some of which likely affect virulence (3). Only limited sequence mapping of Californian MYXV has been undertaken to date. This demonstrated that a number of potential virulence genes were duplicated in the MSW and MSD strains, by an expansion of the TIRs compared to those in Lu. However, no novel genes were identified that could explain the very high virulence of Californian viruses (16). Here we describe the complete genome sequence of the MSW strain of Californian MYXV. A comparative analysis with other MYXV genomes provides a new opportunity to examine the adaptation of the leporipoxviruses to their natural hosts, as well as the mechanisms of MYXV virulence in European rabbits.

MATERIALS AND METHODS

Virus and DNA preparation. MSW:myxoma virus:California/San Francisco 1950 (11) was originally obtained as freeze-dried powdered rabbit tissue from the late Frank Fenner (John Curtin School of Medical Research, Australian National University, Canberra, Australia). The virus was subsequently passaged once in a laboratory rabbit, and testis-derived virus was amplified three times in RK-13 cells to generate seed and working stocks. The third-passage working stock was used to infect 10 T175 flasks of RK-13 cells at a multiplicity of infection (MOI) of approximately 0.075, and virus DNA was prepared as previously described (9).

Sequencing and assembly. Template viral DNA was processed using a TruSeq DNA sample preparation kit (Illumina) to produce a multiplex library for sequencing. Briefly, extracted viral genomic DNA (gDNA) was sheared with a Covaris AFA system, creating fragments of 50 to 7,000 bp; sheared samples were then end repaired, purified, and 3′ adenylated. Barcoded sequencing adapters were ligated, and 400- to 500-bp fragments were purified. After fragment enrichment and cleanup with AMPure XP beads, individual library components were quantitated by quantitative PCR (qPCR), normalized, and pooled into a final sequencing library consisting of 8 different viral genomes (this included seven other South American MYXV strains that were analyzed in a separate study), which was run in a single lane of an Illumina HiSeq2000 to generate 100-bp paired-end reads. De-multiplexed reads were quality trimmed with the trim.pl script (http://wiki.bioinformatics.ucdavis.edu/index.php/Trim.pl) and assembled with the Velvet *de novo* assembler (17), using a k-mer value of 61 and an expected coverage of 2,000×. A single scaffold consisting of two contigs was generated, with homogeneous coverage across the single copy region of the segment. A 28-bp gap between the two contigs was closed by PCR. Only one complete, or nearly complete, copy of the TIR was assembled at either the 5′ or the 3′ end, though up to a full read length of the complementary TIR was observed at the opposite end, allowing easy identification of the TIR junction. To further verify the position of the TIR junction, we duplicated the complete TIR, generated a reverse complement of the sequence that was added on the opposite end, and remapped the sequence reads to that assembled portion of the genome. A subset of 2,000,000 reads was then mapped to the assembly with the added TIR sequence, and the junction of the TIR was inspected for both read pairs that spanned this region, as well as reads in either direction spanning the TIR junction.

Data analysis. Genome annotation was transferred from Lu to MSW by use of the Rapid Annotation Transfer Tool (18) and from previous genome mapping of the partial sequence of MSW (16). EMBL flat files of transferred gene models were then inspected and compared to the Lu reference by using the Artemis comparison tool (19); models were corrected, and new gene models were added where transfer had not occurred. Artemis was used to write out multi-fasta nucleotide-containing entries for each gene from all analyzed genomes. Gene IDs were added based on the location in the MYXV genome, with the direction of transcription indicated by L or R (e.g., *M010L*). Genes in the TIR are identified by L/R (e.g., *M007L/R*). Proteins are identified by the same number as the gene, with the transcription direction omitted (e.g., M010). RFV genes and proteins are prefixed with S rather than M, and the numbering system is syntenic with that of Lu, apart from the duplication caused by the expanded TIR in RFV, such that the *S008.2L/R* gene is equivalent to *M156R* and the *S155R* gene is a truncated form of *S009L* (15). Amino acid sequences for all MSW ORFs were aligned with the orthologous amino acid sequences of Lu (accession number NC_001132) and RFV (accession number AF170722), using Clustal implemented in Bioedit (20); alignments were manually adjusted and percent identity calculated in Bioedit. The uncorrected genetic distance (*P* distance) between Lu and MSW was estimated using MEGA v5 (21).

Analysis of the MSW and SG33 recombinant sequences. The SG33 vaccine strain of MYXV was derived from a French field strain (itself derived from the South American Lausanne strain), but at some stage SG33 or its progenitor recombined with a Californian strain of MYXV [possibly the vaccine derived from the MSD (San Diego 1949) strain] (22). We used the RDP, GENECOV, and Bootscan methods available in the RPD3 package (23; http://darwin.uvigo.es/rdp/rdp.html) to characterize the recombination breakpoints in SG33 compared to the complete genome sequences of MYXV strains MSW, Lu, and SLS and RFV. Default parameters were used in all cases. Phylogenetic trees for each recombinant region detected in the RPD3 analysis (see Results) were then inferred using the maximum likelihood (ML) method available in the PhyML package (version 3.0) (24), and assuming a GTR+Γ model of nucleotide substitution, subtree pruning and regrafting (SPR) branch swapping, and 1,000 bootstrap replications.

For an additional fine-scale screen for recombination breakpoints between sites 78,000 and 83,000 (see Results), we employed a sliding window Bayesian Markov chain Monte Carlo (MCMC) approach. For this analysis, MrBayes trees (25) were generated for 100-bp windows (with a step size of 10 bp), using four chains and 120,000 MCMC generations, with sampling every 50 generations and with five burn-in samples removed. Posterior probabilities for both the SG33/MSW and SG33/Lu+SLS topologies were then plotted in R, using the GGplot2 library.

Nucleotide sequence accession numbers. Sequence data generated in this study have been submitted to GenBank and assigned accession number KF148065.

RESULTS

The MSW genome consists of 164,600 bp of dsDNA with an AT content of 54.56% (compared to 56.4% for Lu and 60.5% for

RFV). This genome is larger than that of Lu (161,777 bp), predominantly due to an expansion of the TIRs (see below). Aside from the TIR expansion, the gene orders in MSW and Lu are identical, with the exception that MSW lacks the *M000.5L/R* ORFs at the extreme ends of the TIRs. There are at least 19,182 (observed) nucleotide substitutions in MSW compared to Lu (*P* distance of 0.12). Based on the Lu sequence, MSW carries 152 intact genes, 15 of which—plus two disrupted ORFs—are duplicated or partially duplicated in the TIRs. In addition, five ORFs present in Lu are disrupted by indels or mutations to the ATG start codon in MSW. Table 1 summarizes the MSW genes and compares the amino acid sequence of each protein with those of its orthologues from Lu and RFV. No novel genes were identified in the MSW sequence compared to Lu.

TIR expansion. The TIRs of MSW consist of 15,464 bp each, compared to 11,577 bp in Lu; each TIR incorporates orthologues of the Lu *M001L/R*, *M002L/R*, *M003.1L/R*, *M003.2L/R*, *M004L/R*, *M004.1L/R*, *M005L/R*, *M006L/R*, *M007L/R*, *M008L/R*, *M156R*, *M154L*, *M153R*, and *M151R* genes and the 3′ 694 bp of the *M150R* gene. Sequences orthologous to the Lu *M008.1L/R* (SERP-1) and *M152R* (SERP-3) ORFs are present in the MSW TIR but have multiple disruptions to the ORFs (see below). Compared to the Lu TIR, 4,216 bp of the *M156R*, *M154L*, *M153R*, *M152R*, and *M151R* sequences and part of the *M150R* sequence from the right-hand (RH) end of the genome have been duplicated at the left-hand (LH) TIR. This duplication has been accompanied by the deletion of 845 nucleotides (nt) of the 3′ end of the *M009L* gene (based on the Lu sequence) and the 23-nt untranslated sequence between the stop codon of *M009L* and the TIR boundary (Fig. 1). The promoter and 5′ 782 bp of the *M150R* gene have not been duplicated, so the TIR boundary now occurs at this point in *M150R* rather than in the *M156R* gene, as in Lu, or the *M009L* gene, as in RFV. A full-length copy of the *M150R* gene is maintained at the RH end of the genome.

Genes disrupted or missing in MSW. Compared to Lu, the following genes are disrupted or missing in MSW. The Lu *M000.5L/R* gene is a predicted ORF encoding 72 amino acids (aa) at the extreme ends of the genome and has an unknown function (13). It is not known if this is a functional gene, and there are no homologues of the putative protein in GenBank. Although there is an ATG codon in MSW at the same location as that of the Lu ATG, there is no ORF and there are multiple large gaps in the alignment.

The Lu *M008.1L/R* gene is the final complete ORF in the Lu TIR, and it encodes a secreted serine proteinase inhibitor (SERP-1) of 369 aa which inhibits the inflammatory response and has a major role in virulence (26, 27). The equivalent nucleotide sequence in MSW retains an ATG start codon and TAA stop codon in equivalent positions, but multiple indels disrupt the ORF. There is a potential ORF encoding 204 aa, starting at the ATG at position 409, with an imperfect late promoter sequence (CC*A*AAATG; italics indicate A rather than the consensus T at −1, and underlining indicates +1 to +4 of the putative promoter). The translated ORF aligns with aa 142 to 268 of Lu M008.1, but after aa 268 the sequence homology is lost.

The Lu *M009L* gene encodes a putative E3 ubiquitin (Ub) ligase of 509 aa with an N-terminal BTB-BACK domain followed by 4 kelch motifs (28). The MSW *M009L* sequence aligns with only the first 685 nucleotides of the Lu sequence; after this, the next 845 nt of *M009L* have been replaced by a sequence from the RH end due to the expansion of the TIR at the LH end of the genome (see above). The ORF, however, encodes only the first 96 aa of M009 due to a stop codon and multiple indels after this point. Most recent Australian isolates of MYXV have a disruption to the *M009L* ORF (9), which suggests that *M009L* is not important for MYXV in either *O. cuniculus* or *S. bachmani*. Lu *M023R* encodes a 61-aa protein of unknown function. However, in MSW, the ATG start codon is mutated to ACG, which also mutates the critical T residue at position +4 in the late promoter structure to a C (from AGT*A*AATG in Lu to AGT*A*AACG in MSW; the promoter from positions −3 to +4 is underlined). Transcription would be predicted to initiate at the A at position +1 (shown in italics), and translation starts at the ATG. In addition, there is a CC insertion at nt 83 and 84, which would disrupt the MSW ORF. An in-frame ATG at nt 19 in Lu is also present in the MSW sequence and would provide a potential ORF encoding 32 aa in MSW, with only 12 amino acids aligning with the Lu sequence. The RFV orthologue, *S023R*, encodes a potential 35-aa protein which is 74% identical to the N-terminal 35-aa sequence of the Lu M023 protein.

M131R encodes an inactive homologue of mammalian Zn/Cu superoxide dismutase (SOD) and is predicted to interfere with the activity of cellular SOD (29–31). Deletion of the *M131R* gene had no effect on the virulence of MYXV in European rabbits. The MSW *M131R* ORF is disrupted by multiple in-frame stop codons.

Finally, Lu *M152R* encodes the 266-aa SERP-3 protein, which is a virulence factor in European rabbits (32). The MSW *M152R* orthologous sequence contains multiple stops in all reading frames.

Genes with major differences between Lu and MSW. Lu *M013L* encodes a 126-aa polypeptide with a pyrin (PYD) domain in the N-terminal 81 aa (33, 34). The MSW M013 protein is only 112 aa due to a deletion after residue 87, but the PYD domain is highly conserved with both Lu and RFV, which has a similar deletion after residue 83 and carries a full-length protein of 107 aa. The LF residues at the C terminus are also conserved in the virus proteins, including that of MSW, but are not present in the PYD domains in the cell proteins POP and ASC (33).

Note that MSW lacks the C-terminal 9 residues of *M030L* (RNA polymerase subunit rpo30; intermediate transcription factor 1) compared to RFV and Lu. Lu *M063R* encodes a host range protein which is required for replication in rabbit cells and European rabbits but is not necessary for replication in primate cell lines (35). The protein has sequence homology to the vaccinia virus (VACV) host range C7 protein and also to the proteins encoded by *M062R* and *M063R*. The MSW orthologue has relatively limited amino acid sequence homology to both M063 of Lu (67.9% identity) and SO63 of RFV (64.3% identity). Like that of RFV, it lacks 9 aa from the highly acidic C-terminal region of M063, with a predicted protein of 201 aa, compared to 215 aa in Lu and 202 aa in RFV. Despite the sequence divergence, all three proteins are compatible with replication in European rabbits.

Lu *M077L* encodes a 143-aa structural protein orthologous to VACV D2. The MSW M077 protein is potentially 23 aa longer at the N terminus due to an alternative in-frame ATG within the *M078R* ORF. D2 forms part of a seven-protein core complex during VACV assembly (36). Interestingly, the MYXV vaccine strain SG33 has the same sequence as MSW for this gene following a recombination event (see below).

M119L encodes a small polypeptide (50 aa) of unknown function. Both MSW and RFV carry smaller potential ORFs, encoding

TABLE 1 Summary of MSW genes and functions and comparison of amino acid sequences with those of orthologues in Lu and RFV

Gene	Location (nt)	Function of gene product	No. of aa		% Identity to Lu protein	No. of RFV aa	% Identity to RFV protein
			MSW	Lu			
M000.5L	Not present	Unknown		72		NA[a]	
M001L	592–1353	Secreted chemokine binding protein	253	260	68.8	258	70.9
M002L	1456–2424	Tumor necrosis factor receptor (TNF-R) homologue	322	326	73.3	325	77.2
M003.1L	2531–2986	VACV B15R	151	151	84.7	151	77.4
M003.2L	3056–3400	Unknown	114	113	80.8	125	79.3
M004L	3629–4342	Apoptosis regulator	237	237	78	225	70
M004.1L	4346–4615	Unknown	89	90	91.1	80	80.8
M005L	4635–6086	E3 Ub ligase	483	483	83.6	484	74.1
M006L	6120–7649	Putative E3 Ub ligase	509	509	77	508	73.4
M007L	7690–8478	Gamma IFN receptor homologue	262	263	83.6	262	69
M008L	8526–10079	Putative E3 Ub ligase	517	515	77.9	514	70.2
M008.1L	Disrupted	Secreted serpin		369		Deleted	
M156L	11134–11367	Interferon resistance; elF2α homologue		Not present in this location		Not present in this location	
M154R	11578–12219	Downregulation of NF-κB? VACV M2L orthologue		Not present in this location		Not present in this location	
M153L	12312–12917	E3 Ub ligase/major histocompatibility complex class 1 (MHC-1) downregulation		Not present in this location		Not present in this location	
M152L	Disrupted	SERP-3		Not present in this location		Not present in this location	
M151L	13754–14755	SERP-2		Not present in this location		Not present in this location	
M150L	Partial sequence (14771–15464)			Not present in this location		Not present in this location	
M009L	Truncated sequence (15465–16151)	Putative E3 Ub ligase		509		510	
M010L	16519–16770	Epidermal growth factor-like protein	83	85	83.5	80	69
M011L	16752–17252	Apoptosis regulator	166	166	71.8	163	74.6
M012L	17268–17717	dUTP nucleotidylhydrolase	149	148	87.2	143	82
M013L	17736–18074	Pyrin domain/inflammasome	112	126	62.5	107	74.1
M014L	18104–19657	Putative E3 Ub ligase	517	517	84.5	516	77.2
M015L	19708–20676	Ribonucleotide reductase small subunit	322	322	95.6	322	94.4
M016L	20705–20938	Unknown	77	77	67.5	82	53.6
M017L	20941–21171	Unknown	76	76	86.8	77	81.8
M018L	21386–21586	VACV F8L	66	66	96.9	66	96.9
M019L	21641–22288	Fusion/entry	215	215	96.2	215	93.4
M020L	22266–23606	Ser/Thr protein kinase	446	446	95.5	445	93.2
M021L	23733–25610	Enveloped virus (EV) maturation	625	625	88.3	627	84.2
M022L	25642–26757	EV protein	371	371	94.8	370	93.5
M023L	Disrupted	Unknown		61		35	
M024L	26928–27374	VACV F15L	148	148	95.2	148	94.5
M025L	27431–28060	VACV F16L	209	209	93.3	209	89.9
M026R	28101–28409	DNA binding	102	102	97	101	88.3
M027L	28406–29818	Poly(A) polymerase (Pol) catalytic subunit	470	470	98.2	470	97.2
M028L	29815–32010	EV formation?	731	731	88.9	721	85.5
M029L	32050–32397	IFN resistance; VACV E3L orthologue	115	115	88.6	116	85.3
M030L	32460–33107	RNA Pol subunit	215	222	86.1	222	83.4
M031R	33208–34392	Virosome protein	394	393	77.4	392	75.8
M032R	34402–36099	VACV E6R	565	565	95.9	565	92.2
M033R	36102–36920	Core protein	272	272	97.7	274	94.8
M034L	36917–39940	DNA Pol	1007	1006	96.2	1005	92.1
M035R	39974–40264	Thiol-oxidoreductase	96	96	90.6	96	89.5
M036L	40285–42327	VACV O1L/Erk1/2 signaling?	680	680	91.3	681	88.1
M037L	42363–42461	VACV 03L/fusion complex	32	32	100	32	93.7
M038L	42472–43413	Core protein	313	313	95.2	313	94.5
M039L	43414–43638	VACV I2L/membrane protein	74	74	83.7	74	89.1
M040L	43639–44448	DNA binding protein	269	270	95.9	270	93.7
M041L	44522–44758	Structural protein	78	78	96.1	78	83.3
M042L	44779–45939	Core protein	386	386	92.2	386	91.1
M043L	45932–47221	Core protein	429	429	97.6	429	96.2
M044R	42227–49263	RNA helicase/nucleophosphohydrolase	678	678	90.7	678	88
M045L	49260–51032	Core enzyme	590	590	90.3	590	86.4
M046L	51029–51364	Fusion/entry	111	111	94.5	111	94.5
M047R	51358–52035	Elongation factor/late transcription	225	225	92.8	225	91.1
M048L	51993–52382	Glutaredoxin 2 homologue	129	128	96.1	128	95.3
M049R	52385–53680	Core protein	431	431	85.3	432	84
M050R	53682–53873	RNA Pol subunit	63	63	93.6	63	96.8
M051R	53873–54394	VACV G6R	173	174	93.6	174	89.6
M052L	54363–55415	Core protein	350	350	94.2	350	90.5
M053R	55445–56227	Late transactivator protein	260	260	98.8	260	99.2
M054R	56247–57245	Fusion complex	332	332	93.9	332	92.7
M055R	57246–57974	Structural protein	242	242	99.1	242	97.1
M056R	58030–58329	VACV L2R	99	99	61.6	99	66.6

(Continued on following page)

TABLE 1 (Continued)

Gene	Location (nt)	Function of gene product	No. of aa		% Identity to Lu protein	No. of RFV aa	% Identity to RFV protein
			MSW	Lu			
M057L	58279–59241	Core protein	320	320	91.8	320	92.8
M058R	59266–60021	Major core protein	251	251	97.6	251	97.2
M059R	60040–60429	Fusion/entry	129	129	98.4	129	94.5
M060R	60383–60829	Structural protein	148	148	93.9	148	90.5
M061R	60860–61396	Thymidine kinase	178	178	89.8	178	83.7
M062R	61474–61947	Host range protein	157	158	83.5	158	74.6
M063R	62004–62609	Host range protein	201	215	67.9	202	64.3
M064R	62655–63254	Host range protein	199	203	76.5	198	64.1
M065R	63296–64312	Poly(A) Pol subunit	338	338	98.5	338	96.4
M066R	64212–64769	RNA Pol subunit	185	185	97.2	185	93.5
M067L	64772–65173	Fusion complex	133	133	98.4	133	95.4
M068R	65247–69107	RNA Pol subunit	1286	1286	97.8	1286	97.9
M069L	69115–69633	Tyr/Ser phosphatase	172	172	98.8	173	95.3
M070R	69649–70221	Fusion complex	190	190	98.4	190	97.3
M071L	70224–71198	Structural protein	324	324	94.4	324	89.1
M072L	71199–73589	RNA Pol-associated transcription factor	796	796	95.4	798	95.1
M073R	73719–74303	Late transactivator protein	194	194	88.7	198	82.4
M074R	74331–75278	DNA topoisomerase 1	315	315	94.9	314	95.8
M075R	75278–75721	VACV H7R	147	147	95.9	150	89.3
M076R	75723–78230	mRNA capping enzyme subunit	835	835	93.2	836	91.8
M077L	78192–78692	Structural protein	166	166	80.7	166	72.2
M078R	78629–79351	Structural protein	240	240	91.2	241	82.9
M079R	79348–80004	Uracil-DNA glycosylase	218	218	96.7	218	94.9
M080R	80038–82398	Nucleoside triphosphatase	786	786	97.3	786	96
M081R	82395–84302	Early transcription factor subunit	635	635	97.9	635	97.9
M082R	84335–84826	RNA Pol subunit	163	163	95	163	95.7
M083L	84797–85654	Carbonic anhydrase homologue/structural protein?	285	286	90.5	285	87.3
M084R	85703–86323	VACV D9R/MutT-like protein	206	206	91.7	206	93.2
M085R	86320–87099	VACV D10R/MutT-like protein	259	259	88.4	260	88.8
M086L	87102–89000	Nucleoside triphosphatase 1/DNA helicase	632	632	93.9	632	92.5
M087L	89018–89881	mRNA capping enzyme, small subunit/VITF	287	287	97.9	287	97.2
M088L	89910–91571	Intracellular mature virion (IMV) virion protein	553	554	96	552	94.3
M089L	91598–92047	Late transcription factor 2	149	149	93.9	149	94.6
M090L	92081–92755	Late transcription factor 3	224	224	99.1	224	99.1
M091L	92752–92979	Thiol oxido reductase	75	75	98.6	75	94.6
M092L	92988–94946	Core protein	652	653	96.9	653	94.6
M093L	94984–95454	Core protein	156	159	79.8	153	69.2
M094R	95494–95988	RNA Pol subunit	164	164	97.5	164	95.1
M095L	95985–97106	Core protein	373	373	93.2	373	94.1
M096L	97134–99269	Early transcription factor subunit	711	711	96.4	711	95.7
M097R	99322–100182	Intermediate transcription factor subunit	286	286	95.1	286	93.3
M098L	100183–100416	Membrane protein	77	76	96.1	77	94.8
M099L	100417–103122	Core protein precursor	901	901	93.5	902	90.1
M100R	103137–104078	Scaffolding protein	313	313	95.8	314	95.5
M101L	104075–104563	Virion protein	162	161	93.2	156	88.8
M102L	104596–104802	IMV membrane protein	68	68	91.1	68	77.9
M103L	104856–105149	IMV membrane protein	97	96	95.8	95	91.7
M104L	105166–105327	Potential immunomodulatory protein?	53	53	96.2	53	92.4
M105L	105317–105601	Core protein	94	94	94.6	94	91.4
M106L	105585–106715	Fusion complex	376	376	93.3	376	92.8
M107L	106731–107339	IMV membrane protein	202	200	87.6	199	81.1
M108R	107354–108790	DNA helicase/negative transcriptional regulator	478	478	93	478	91
M109L	108771–108992	VACV A19L	73	73	95.8	73	94.5
M110L	108993–109334	Core protein	113	113	95.5	113	89.3
M111R	109333–110628	DNA Pol	431	431	91.6	432	86.1
M112R	110591–111094	Holliday junction resolvase	167	164	85.9	160	82.6
M113R	111101–112255	Intermediate transcription factor subunit	384	385	95	385	93.5
M114R	112281–115748	RNA Pol subunit	1155	1155	98.2	1155	97.1
M115L	115749–116330	Fusion protein/EV formation/IMV surface protein	193	188	73.2	179	75.7
M116L	116331–116753	IMV membrane protein	140	140	100	140	94.2
M117L	116759–117667	RNA Pol subunit	302	302	94	302	92.3
M118L	117636–117866	Core protein	76	76	94.7	76	93.3
M119L	117882–118004	Unknown	40	50	65	39	65
M120L	118038–118805	ATPase	255	255	97.2	255	96.4
M121R	118890–119420	EV glycoprotein/NK receptor homologue	176	176	81.2	172	75
M122R	119427–119942	EV glycoprotein/NK receptor homologue	171	172	94.7	171	91.8
M123R	119976–120515	VACV A35R	179	179	88.8	179	85.4
M124R	120551–121411	Unknown	286	286	86.7	286	87.4
M125R	121425–121928	Unknown	167	161	81.4	164	70.1
M126R	121970–122785	VACV A37R/structural protein?	271	271	92.2	271	88.1
M127L	122772–124109	Photolyase	445	445	88.3	445	84.7
M128L	124112–124960	CD47 homologue	282	281	86.8	290	73.2
M129R	124959–125369	VACV E7R	136	136	88.9	78	46.3

(Continued on following page)

TABLE 1 (Continued)

Gene	Location (nt)	Function of gene product	No. of aa		% Identity to Lu protein	No. of RFV aa	% Identity to RFV protein
			MSW	Lu			
M130R	125440–125823	Unknown	127	122	77.9	104	71.6
M131R	Disrupted	Cu/Zn superoxide dismutase homologue		163		163	
M132L	126359–126892	Unknown	177	175	85.8	175	82.5
M133R	126974–128653	DNA ligase	559	563	91.4	559	89.6
M134R	128772–134765	Variola virus B22R orthologue	1997	2000	85	1939	81.5
M135R	134768–135313	Immunomodulatory protein	181	178	74.5	Deleted	
M136R	135496–136041	Homologue of VACV A52; Bcl-2-like fold	181	179	83.9	Deleted	
M137R	136042–136974	VACV A51R	310	310	84.5	218	52.2
M138L	137003–137875	Alpha-2,3 sialyltransferase	290	290	81.3	290	81.3
M139R	137926–138492	Homologue of VACV A52; Bcl-2-like fold	188	188	93	Deleted	
M140R	138495–140156	Putative E3 Ub ligase	553	553	87.1	553	82.4
M141R	140197–140874	OX-2 homologue	225	218	75.7	223	67
M142R	140882–141814	Ser/Thr protein kinase	310	306	91.6	306	90.3
M143R	141815–142519	RING-E3 Ub ligase	234	234	94.8	234	90.5
M144R	142566–143462	VACV B5R	298	300	76.6	299	75
M146R	143486–143812	VACV N1L orthologue/Bcl-2-like fold	108	108	84.2	108	84.2
M147R	143863–144726	Ser/Thr protein kinase	287	288	88.8	287	86.4
M148R	144792–146816	Putative E3 Ub ligase	674	675	67.6	673	80.4
M149R	146818–148290	Putative E3 Ub ligase	490	490	87.1	490	83.4
M150R	148355–149830	E3 Ub ligase; NF-κB inhibition	491	493	78.5	Deleted	
M151R	149846–150847	SERP-2	333	333	86.1	333	81.6
M152R	Disrupted	SERP-3		266		Deleted	
M153R	151684–152289	E3 Ub ligase/MHC-1 downregulation	201	206	63.5	201	61.8
M154L	152382–153023	Downregulation of NF-κB? VACV M2L orthologue	213	214	89.2	214	84.1
M156R	153234–153467	Interferon resistance; elF2α homologue	77	102	53.3	78	65.3

[a] NA, not applicable.

40 and 39 residues, respectively, that are truncated at the N terminus compared to that in Lu due to initiating at an ATG 30 nucleotides downstream of the *M119L* ATG.

M156R is an orthologue of the VACV *K3L* gene. Like K3, M156 is phosphorylated by cellular protein kinase R (PKR) and is predicted to compete with eIF2α for phosphorylation by PKR, thus inhibiting translational shutdown in response to type 1 interferon (IFN) (37). MSW M156 and the orthologue in RFV, S008.2, lack the first 27 aa of the Lu sequence, initiating from a downstream ATG compared to the case in Lu. This extended N-terminal sequence in Lu is also not present in the orthopoxvirus orthologues of M156, such as K3L. All the other critical binding residues are conserved between Lu and MSW; Y54, which is conserved between Lu, the orthopoxviruses, and swinepox virus, is an H in

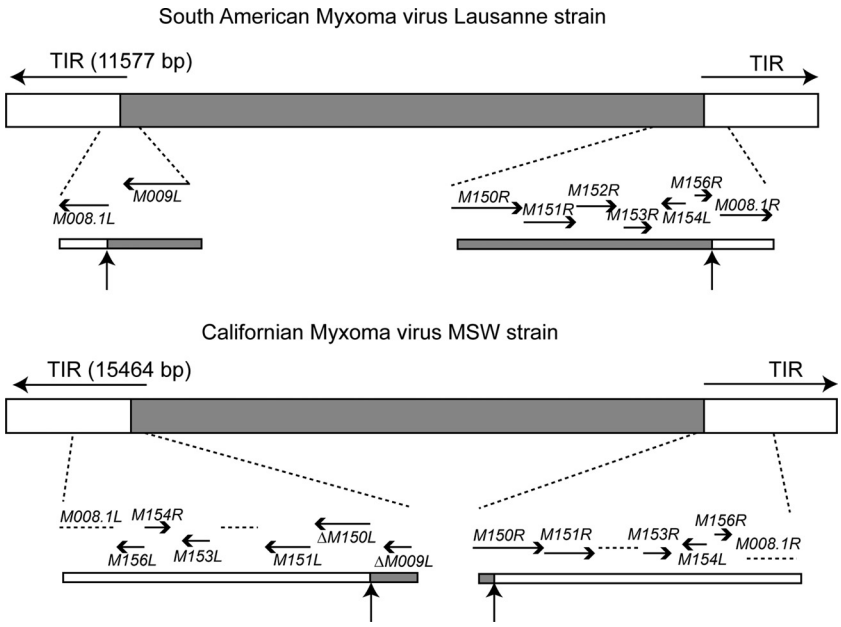

FIG 1 Expansion of the TIR regions of MSW compared to Lu. The diagram shows a representation of the gene order around the TIR boundary at the LH and RH ends of the genome for Lu compared to the same regions for MSW. The vertical arrows indicate the TIR junctions. Note that *M008.1L/R* is labeled in the MSW diagram for clarity but that it is not present as an ORF in MSW and hence is depicted as a dashed line rather than an arrow. The *M152L/R* sequence also does not comprise an ORF in MSW and is shown as a dashed line between *M151L/R* and *M153L/R* but not labeled. The designations Δ*M150L* and Δ*M009L* represent the truncated forms of these ORFs in MSW. *M155* was not used in the MYXV annotation. The figure is not drawn to scale.

```
MSW 153146  GTAAATAATCGTTACACCAGGGCCGTCGAATTGTCCGAAGCCCAGTAAAAACAAAAATATAAAAGTAAATACGTATAGAGCGAGCGCCATG
Lu  149997  GTAAATGACAGTCA---TAAAACCATCGAGTCGTCCCAGGCCGAGGAAAAACAAAAATATAAAAGTAAATACATACAGAACGAGCGCCATG
                  M  T  V     I  K  P  S  S  R  P  R  P  R  K  N  K  N  I  K  V  N  T  Y  R  T  S  A  M
RFV 148928  ATCGTACATAATACAGTAGGAATTTTGTCATGAACCTCTCTTGTCGAGGAAAACATAAATTAAAACAAGTGTATATAGAACGAGCGCTATG
```

FIG 2 Promoter structure and upstream untranslated regions of *M156R* genes from MSW, Lu, and RFV. The nucleotide sequences immediately upstream of the *M156R*, MSW *M156R*, and RFV *S008.2R* genes are shown. The postulated Lu start codon at position 150,001 is shown in italics, while the potential late promoter sequence incorporating this ATG is in a box. The ATG for MSW and RFV and the potential downstream ATG for Lu are shown in bold at the 3′ ends of the sequences. The potential early promoter sequences are underlined, and the TAAAT motifs proximal to these sequences are highlighted by boxes. The N-terminal amino acid sequence of the Lu protein is shown under the nucleotide sequence. The RFV sequence upstream of *S008.1R* is considerably diverged, and no attempt was made to align it with the Lu and MSW sequences.

MSW. As noted above, due to the expansion of the TIRs, *M156R* is duplicated in MSW.

The promoter usage predicted for Lu and RFV is also interesting, with Lu *M156R* predicted to be under the control of a late promoter, albeit one lacking the upstream T/A-rich region, and rabbit fibroma virus *S008.2L/R* predicted to be an early gene (13, 15). The upstream sequence for MSW and RFV and the N-terminal Lu coding sequence are shown in Fig. 2. The sequence around the late promoter is conserved between Lu and MSW, but MSW has an A instead of a G (Lu sequence, CGTAAATG; and MSW sequence, CGTAAATA) and no downstream ATG for another 84 nt. It seems likely that MSW *M156R* is also an early gene, although there is a late promoter motif (AGTAAATA) 19 nt upstream of the ATG. Systematic mutational analysis of poxvirus early promoter sequences showed that inclusion of late promoter motifs could lead to late transcription under some circumstances (38), so it is possible that this gene is expressed constitutively. A TAAAT motif is also present 29 nt upstream of the RFV ATG (Fig. 2). Interestingly, Lu, RFV, and MSW all have an early transcription termination signal (T_5NT) at the 3′ end of the gene. It would seem logical for proteins inhibiting type 1 IFN action to be expressed early, as are the *M029L* gene and VACV *K3L*. Since the sequences of the potential early promoter region upstream of the MSW ATG start codon are identical between MSW and Lu (Fig. 2), it is possible that two transcripts are being produced in Lu: an early transcript that lacks the N-terminal 27 aa, which are not predicted to contribute to the β-barrel structure of M156 (37), and a potential longer late transcript.

Relationship of MSW to the recombinant French SG33 vaccine strain of MYXV. MSW has genomic regions exhibiting strong sequence similarity with the SG33 vaccine strain of MYXV, derived from a French field strain by repeated passages in rabbit kidney cells and chicken embryo fibroblasts (39). The resulting virus (SG33) appears to have recombined with a Californian virus, probably the attenuated vaccine strain derived from MSD (40, 41). This recombinant has a 13.5-kb deletion at the RH end of the genome encompassing multiple genes associated with virulence and significantly truncating the TIRs (22).

Using a variety of methods, we detected significant ($P < 0.05$) recombination breakpoints at nucleotides 78413, 81576, and 136691 in our genome sequence alignment, such that the complete genome alignment of Lu, MSW, SG33, SLS, and RFV could be divided into four distinct regions with differing evolutionary histories (Fig. 3). For regions (nt) 1 to 78412 and 81576 to 136690, which comprise the majority of the genome, SG33 appears to be a close sister group of the Lu and SLS strains of MYXV, originally sampled in South America. In contrast, SG33 is more closely related (and with strong bootstrap support) to MSW in genomic regions 78413 to 81575 and 136691 to 168149, such that SG33 is clearly a recombinant of Californian and South American myxoma viruses, although with multiple breakpoints. RFV was the most divergent lineage, and hence can be assumed to represent an outgroup. A sliding Bayes analysis within the central genomic region (nt 77,000 to 83,000) (Fig. 4) revealed a number of highly localized recombination events between MSW and SG33. Interestingly, the genes located in this region of microrecombination (*M076R*, *M077L*, *M078R*, *M079R*, and *M080R*) have not been dis-

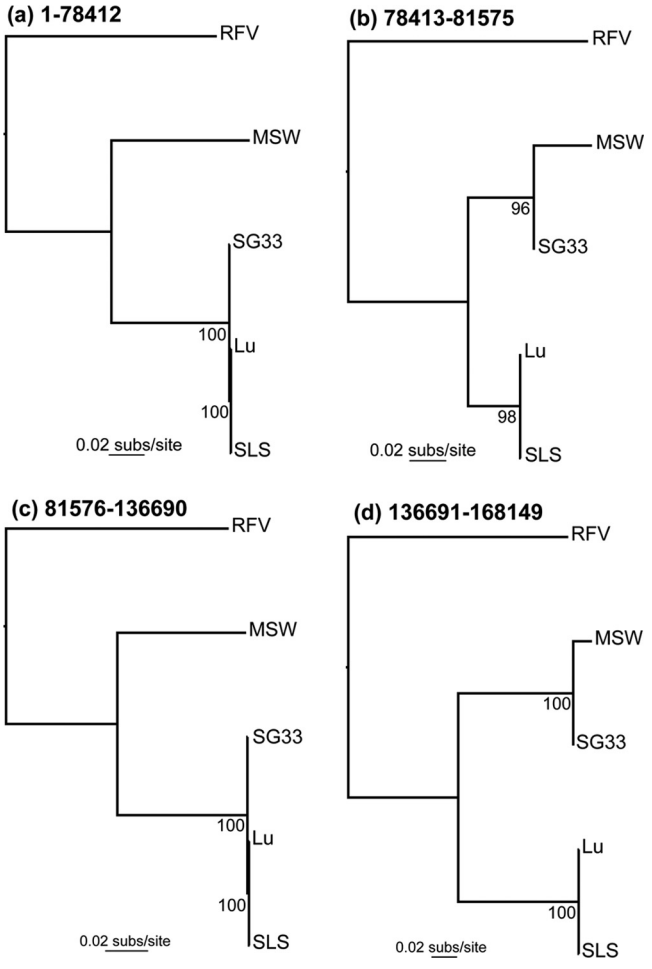

FIG 3 Recombination in the evolutionary history of MYXV. Separate ML trees were estimated for multiple-sequence alignment of regions 1 to 78412 (a), 78413 to 81575 (b), 81576 to 136690 (c), and 136691 to 168149 (d), and the locations of the recombination breakpoints identified in the RDP3 analysis are denoted. In all cases, bootstrap support values are shown for key nodes, and all horizontal branch lengths are scaled according to the number of nucleotide substitutions per site.

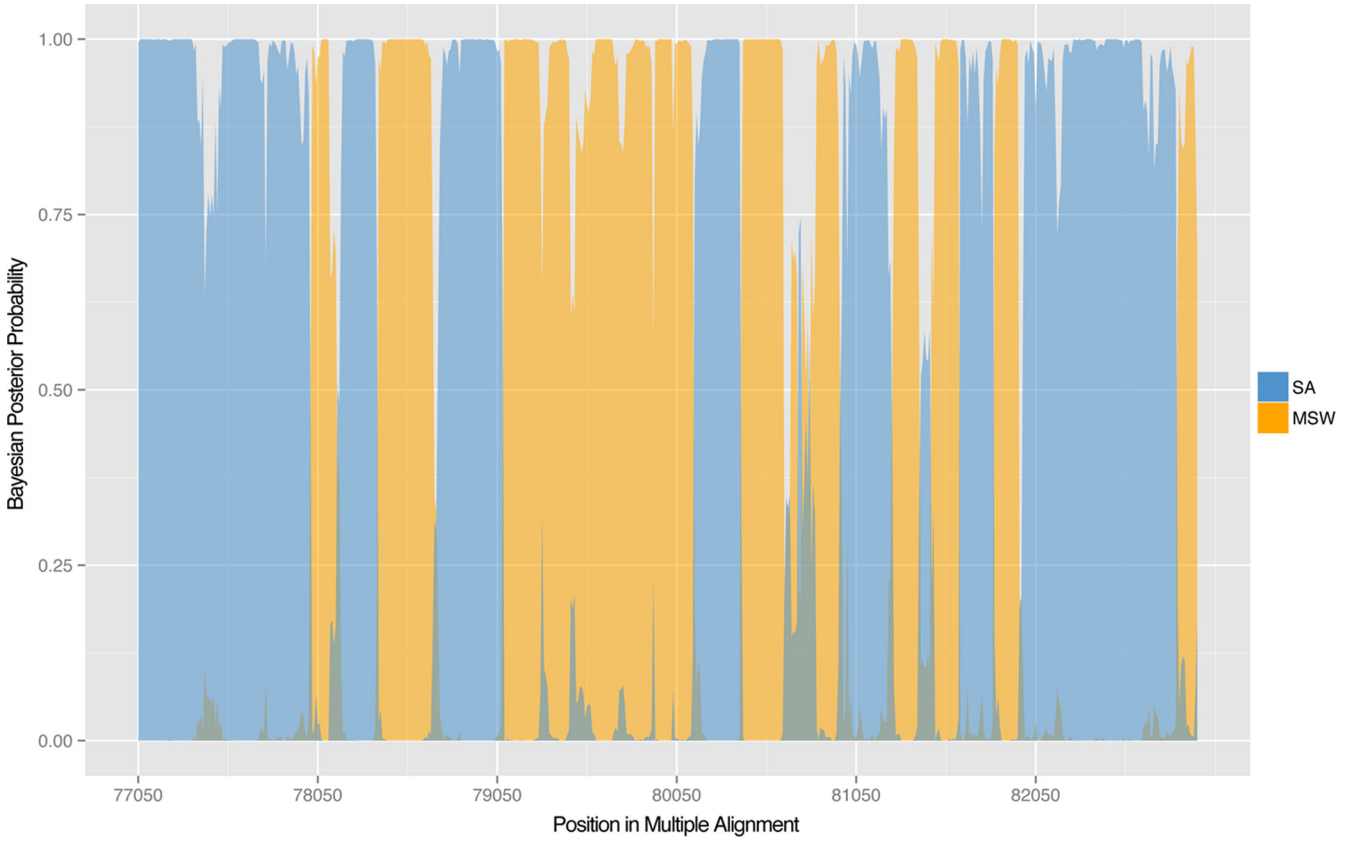

FIG 4 Bayesian posterior probability support for sliding 100-bp windows between positions 77,000 and 83,000 in the multiple-sequence alignment (Fig. 3). The blue area indicates support for a grouping of SG33 with Lu and SLS, while the yellow area indicates support for an SG33/MSW grouping, indicative of microrecombination events.

rupted. Why most recombination events are limited to this 5-kb region is currently unknown. In addition, it is both notable and puzzling that Lu and SLS form a sister group to the exclusion of SG33, even though SG33 was derived from Lu. Whether this is the result of additional highly localized recombination or selective events is unclear and merits further investigation. A similarly complex laboratory recombination event occurred between RFV and a South American MYXV to form malignant rabbit virus (MRV) (42, 43).

DISCUSSION

Our analysis of the complete genome sequence of the MSW strain of Californian MYXV reveals that it is more closely related to the South American MYXV than is the leporipoxvirus RFV and that it acts as a parental strain in a recombination event involving the SG33 vaccine strain of MYXV. However, despite this phylogenetic association, no novel genes were identified that could explain the high virulence of Californian viruses in European rabbits. Indeed, MSW has five ORFs disrupted compared to the Lu strain of MYXV and is missing the *M000.5L/R* ORF. In comparison, RFV has six fragmented genes and is missing *M000.5L/R*, while *S023R* and *S129R* are truncated. Although containing multiple indels, the disrupted ORFs in MSW can readily be aligned with the orthologous sequences in Lu. By comparison, RFV has lost substantially more of its fragmented genes, with relatively little remaining sequence in some cases, which is compatible with a longer period of divergence from the MYXV strains. In addition, RFV contains an expansion of the TIR boundary compared to Lu, with duplication of the *M156R-S008.2L/R* genes and partial duplication of the *S009L* gene. The mechanism determining the length of the TIR region in poxviruses is not understood, but it seems likely that expansion and contraction of the TIR boundary can occur readily, and it is difficult to predict which genes the ancestral leporipoxvirus TIR might have included. A short TIR is compatible with virus viability; for example, SG33 has a severely truncated TIR due to deletion of 13.5 kb at the RH end of the genome (22). Similarly, variola virus, an orthopoxvirus and the causative agent of human smallpox, carries no genes in its short 725-bp TIRs (44).

Four of the genes missing or truncated in RFV are also missing or disrupted in MSW: *M000.5L/R*, *M008.1L/R*, *M152R*, and *M023R*. This suggests that these genes were either redundant or selected against in their respective *Sylvilagus* host species or an earlier ancestral host. Lu appears to have retained a full complement of genes compared to the other two leporipoxviruses, with no obvious fragmented ORFs. Although there are several long intergenic regions that may once have included ORFs, most of the genes have very little intervening sequence. The region containing *M000.5L/R* in Lu does not align well with that in MSW, and it is not clear if this potential gene has been lost, was never present and so may have evolved in South American MYXV, or indeed may not be a functional gene. All of the disrupted ORFs in MSW contain multiple mutations suggesting that they are nonfunctional.

MSW has extreme virulence for European rabbits, based on its shorter average survival time than that of Lu, minimal clinical signs, and ability to overcome strong genetic resistance to MYXV in Australian wild rabbits (11, 12). There are no novel genes that might explain this high virulence, and two genes that in the Lu strain are each critical for virulence in European rabbits, *M008.1L/R* and *M152R*, have been lost in MSW. This suggests either that the duplication of *M156R*, *M154L*, *M153R*, and *M151R*—which include two known virulence genes and two likely immune modulator genes whose effect on virulence has not been determined—is able to overcome the loss of the *M008.1L/R* and *M152R* genes because of increased expression of the encoded immunomodulatory proteins or that sequence differences in other key proteins are responsible for even more efficient suppression of the European rabbit innate and adaptive immune responses than that caused by the South American virus. This has presumably been due to the coevolution with *S. bachmani*, which has selected gene variants that suppress the immune response to enable replication to high titers in localized fibromas. The South American viruses, however, were unable to reach transmissible titers in *S. bachmani*, and the Californian virus did not cause lesions in *S. brasiliensis*, even though both viruses are lethal in European rabbits (5, 45).

ACKNOWLEDGMENTS

This work was funded in part by grant R01 AI093804 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health. E.C.H. was supported by an NHMRC Australia Fellowship, and D.C.T. was supported by an ARC Future Fellowship.

REFERENCES

1. **Fenner F, Ratcliffe FN.** 1965. Myxomatosis. Cambridge University Press, Cambridge, United Kingdom.
2. **Luna RML.** 2000. First report of myxomatosis in Mexico. J. Wildl. Dis. **36:**580–583.
3. **Kerr PJ.** 2012. Myxomatosis in Australia and Europe: a model for emerging infectious diseases. Antiviral Res. **93:**387–415.
4. **Spiesschaert B, McFadden G, Hermans K, Nauwynck H, Van de Walle GR.** 2011. The current status and future directions of myxoma virus, a master in immune evasion. Vet. Res. **42:**76.
5. **Regnery DC.** 1971. The epidemic potential of Brazilian myxoma virus (Lausanne strain) for three species of North American cottontails. Am. J. Epidemiol. **94:**514–519.
6. **Regnery DC, Marshall ID.** 1971. Studies in the epidemiology of myxomatosis in California. IV. The susceptibility of six leporid species to Californian myxoma virus and the relative infectivity of their tumors for mosquitoes. Am. J. Epidemiol. **94:**508–513.
7. **Alda F, Gaitero T, Suarez M, Doadrio I.** 2009. Molecular characterisation and recent evolution of myxoma virus in Spain. Arch. Virol. **154:**1659–1670.
8. **Dalton KP, Nicieza I, Baragano A, Alonso JM, Parra F.** 2010. Molecular characterisation of virulence graded field isolates of myxoma virus. Virol. J. **7:**49. doi:10.1186/1743-422X-7-49.
9. **Kerr PJ, Ghedin E, Depasse JV, Fitch A, Cattadori IM, Hudson PJ, Tscharke DC, Read AF, Holmes EC.** 2012. Evolutionary history and attenuation of myxoma virus on two continents. PLoS Pathog. **8:**e1002950. doi:10.1371/journal.ppat.1002950.
10. **Kessel JF, Fisk RT, Prouty CC.** 1934. Studies with the Californian strain of the virus of infectious myxomatosis, p 2927–2939. *In* Proceedings of the Fifth Pacific Science Congress. University of Toronto Press, Toronto, Canada.
11. **Fenner F, Marshall ID.** 1957. A comparison of the virulence for European rabbits (*Oryctolagus cuniculus*) of strains of myxoma virus recovered in the field in Australia, Europe and America. J. Hyg. **55:**149–191.
12. **Silvers L, Inglis B, Labudovic A, Janssens PA, van Leeuwen BH, Kerr PJ.** 2006. Virulence and pathogenesis of the MSW and MSD strains of Californian myxoma virus in European rabbits with genetic resistance to myxomatosis compared to rabbits with no genetic resistance. Virology **348:**72–83.
13. **Cameron C, Hota-Mitchell S, Chen L, Barrett J, Cao JX, Macaulay C, Willer D, Evans D, McFadden G.** 1999. The complete DNA sequence of myxoma virus. Virology **264:**298–318.
14. **Morales M, Ramirez MA, Cano MJ, Parraga M, Castilla J, Perez-Ordoyo LI, Torres JM, Barcena J.** 2009. Genome comparison of a nonpathogenic myxoma virus field strain with its ancestor, the virulent Lausanne strain. J. Virol. **83:**2397–2403.
15. **Willer DO, McFadden G, Evans DH.** 1999. The complete genome sequence of Shope (rabbit) fibroma virus. Virology **264:**319–343.
16. **Labudovic A, Perkins H, van Leeuwen B, Kerr P.** 2004. Sequence mapping of the Californian MSW strain of myxoma virus. Arch. Virol. **149:**553–570.
17. **Zerbino DR, McEwen GK, Margulies EH, Birney E.** 2009. Pebble and rock band: heuristic resolution of repeats and scaffolding in the velvet short-read de novo assembler. PLoS One **4:**e8407. doi:10.1371/journal.pone.0008407.
18. **Otto TD, Dillon GP, Degrave WS, Berriman M.** 2011. RATT: Rapid Annotation Transfer Tool. Nucleic Acids Res. **39:**e57. doi:10.1093/nar/gkq1268.
19. **Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J.** 2005. ACT: the Artemis Comparison Tool. Bioinformatics **21:**3422–3423.
20. **Hall TA.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41:**95–98.
21. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28:**2731–2739.
22. **Camus-Bouclainville C, Gretillat M, Py R, Gelfi J, Guerin JL, Bertagnoli S.** 2011. Genome sequence of SG33 strain and recombination between wild-type and vaccine myxoma viruses. Emerg. Infect. Dis. **17:**633–638.
23. **Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P.** 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics **26:**2462–2463.
24. **Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O.** 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. **59:**307–321.
25. **Ronquist F, Huelsenbeck JP.** 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:**1572–1574.
26. **Macen JL, Upton C, Nation N, McFadden G.** 1993. SERP1, a serine proteinase inhibitor encoded by myxoma virus, is a secreted glycoprotein that interferes with inflammation. Virology **195:**348–363.
27. **Upton C, Macen JL, Wishart DS, McFadden G.** 1990. Myxoma virus and malignant rabbit fibroma virus encode a serpin-like protein important for virus virulence. Virology **179:**618–631.
28. **Zhang L, Villa NY, McFadden G.** 2009. Interplay between poxviruses and the cellular ubiquitin/ubiquitin-like pathways. FEBS Lett. **583:**607–614.
29. **Cao JX, Teoh ML, Moon M, McFadden G, Evans DH.** 2002. Leporipoxvirus Cu-Zn superoxide dismutase homologs inhibit cellular superoxide dismutase, but are not essential for virus replication or virulence. Virology **296:**125–135.
30. **Teoh ML, Turner PV, Evans DH.** 2005. Tumorigenic poxviruses upregulate intracellular superoxide to inhibit apoptosis and promote cell proliferation. J. Virol. **79:**5799–5811.
31. **Teoh ML, Walasek PJ, Evans DH.** 2003. Leporipoxvirus Cu,Zn-superoxide dismutase (SOD) homologs are catalytically inert decoy proteins that bind copper chaperone for SOD. J. Biol. Chem. **278:**33175–33184.
32. **Guerin JL, Gelfi J, Camus C, Delverdier M, Whisstock JC, Amardeihl MF, Py R, Bertagnoli S, Messud-Petit F.** 2001. Characterization and functional analysis of Serp3: a novel myxoma virus-encoded serpin involved in virulence. J. Gen. Virol. **82:**1407–1417.
33. **Dorfleutner A, Talbott SJ, Bryan NB, Funya KN, Rellick SLRJC, Shi X, Rojanasakul Y, Flynn DC, Stehlik C.** 2007. A Shope fibroma virus PYRIN-only protein modulates the host immune response. Virus Genes **35:**685–694.
34. **Johnston JB, Wang G, Barrett JW, Nazarian SH, Colwill K, Moran M, McFadden G.** 2005. Myxoma virus M-T5 protects infected cells from the stress of cell cycle arrest through its interaction with host cell cullin-1. J. Virol. **79:**10750–10763.

35. **Barrett JW, Shun Chang C, Wang G, Werden SJ, Shao Z, Barrett C, Gao X, Belsito TA, Villenevue D, McFadden G.** 2007. Myxoma virus M063R is a host range gene essential for virus replication in rabbit cells. Virology **361:**123–132.
36. **Condit RC, Moussatche N, Traktman P.** 2006. In a nutshell: structure and assembly of the vaccinia virion. Adv. Virus Res. **66:**31–124.
37. **Ramelot TA, Cort JR, Yee AA, Liu F, Goshe MB, Edwards AM, Smith RD, Arrowsmith CH, Dever TE, Kennedy MA.** 2002. Myxoma virus immunomodulatory protein M156R is a structural mimic of eukaryotic translation initiation factor eIF2alpha. J. Mol. Biol. **322:**943–954.
38. **Davison AJ, Moss B.** 1989. Structure of vaccinia virus early promoters. J. Mol. Biol. **210:**749–769.
39. **Saurat P, Gilbert Y, Ganiere J-P.** 1978. Etude d'une souche de virus myxomateux modifée. Rev. Med. Vet. **129:**415–451.
40. **McKercher DG, Saito JK.** 1964. An attenuated live virus vaccine for myxomatosis. Nature **202:**933–934.
41. **Saito JK, McKercher DG, Castrucci G.** 1964. Attenuation of the myxoma virus and use of the living attenuated virus as an immunizing agent for myxomatosis. J. Infect. Dis. **114:**417–428.
42. **Block W, Upton C, McFadden G.** 1985. Tumorigenic poxviruses: genomic organization of malignant rabbit virus, a recombinant between Shope fibroma virus and myxoma virus. Virology **140:**113–124.
43. **Upton C, Macen JL, Maranchuk RA, DeLange AM, McFadden G.** 1988. Tumorigenic poxviruses: fine analysis of the recombination junctions in malignant rabbit fibroma virus, a recombinant between Shope fibroma virus and myxoma virus. Virology **166:**229–239.
44. **Massung RF, Liu LI, Qi J, Knight JC, Yuran TE, Kerlavage AR, Parsons JM, Venter JC, Esposito JJ.** 1994. Analysis of the complete genome of smallpox variola major virus strain Bangladesh-1975. Virology **201:**215–240.
45. **Marshall ID, Regnery DC.** 1963. Studies in the epidemiology of myxomatosis in California. III. The response of brush rabbits (*Sylvilagus bachmani*) to infection with exotic and enzootic strains of myxoma virus and the relative infectivity of the tumours for mosquitoes. Am. J. Hyg. (Lond.) **1963:**213–219.