The Detection and Variation of Strawberry mottle virus

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ABSTRACT

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Strawberry mottle virus (SMoV) is probably the most important virus to infect strawberry (*Fragaria* spp.). All species of strawberry are susceptible to SMoV, resulting in severe losses both in fruit and runner yield. However, due to the absence of definitive symptoms in commercial varieties, the only effective means of detecting SMoV is by transmission to susceptible indicator plants. In this study, we describe a reverse transcription-polymerase chain reaction (RT-PCR) method for the detection of SMoV in *Fragaria* spp. with the use of primers specific for the noncoding regions (NCR) of both RNA1 and RNA2 of the virus. Using this method, all of 16 isolates from various geographical origins were positive for SMoV. Partial sequences of a 327-nt long coding region were obtained for the putative large coat protein of all isolates by RT-PCR using degenerate primers. Nucleotide identities between isolates ranged from 72.8 to 99.7%. A 546-nt sequence of the putative polymerase gene of nine isolates was obtained by RT-PCR and compared. Nucleotide identities ranged from 73.4 to 100%. There was a clear tendency for isolates to group according to their geographical origin. Sequence data obtained of the NCR show four completely conserved regions of 20 or more bases.

Additional keywords: CLUSTAL X, conserved amino acid motifs, extraction method, phylogenetic analysis

Strawberry mottle virus (SMoV) is the most economically important virus to infect strawberries (*Fragaria* spp.), both in terms of its geographical distribution and in the potential loss in yield it can cause. Reductions in fruit yield and runner production in single infections can be as high as 30%. In mixed infections with one or more of the other main strawberry infecting aphid-borne viruses, *Strawberry mild yellow edge virus* (SMYEV), *Strawberry crinkle virus* (SCV), and *Strawberry vein banding virus* (SVBV), losses can be up to 80% (3,12). The classification of SMoV, as a virus species, was until the 1950s often vague due to the similarities in the symptoms it induced in *Fragaria* spp. with those of SCV. The names "Mild crinkle" (20) and "virus I" (19) were eventually superseded by "mottle" (24). Nevertheless, a clear distinction between SMoV and SCV was made when the virus-vector relationships were clarified: SMoV is transmitted in a semipersistent manner, and SCV in a persistent manner. Attempts to further characterize SMoV had limited success because of difficulties in purifying the virus from infected plants, including her-

baceous hosts. Isolation of two double-stranded RNAs and isometric particles of approximately 30 nm in diameter in SMoV-infected plants was confirmed in various independent reports (6,8,9,28). Recently, the complete nucleotide sequence of SMoV was elucidated (26). In genomic organization, it is closest to nepoviruses (11); however, because of its aphid transmissibility and its close similarity to Satsuma dwarf virus (SDV) and related viruses, it has been tentatively ascribed to a separate virus group of SDV-like lineage of picorna-like viruses (5).

All species of *Fragaria* are susceptible to SMoV, although identification of infection, particularly in commercial varieties, is difficult due to the absence of any defining leaf symptoms. Therefore, grafting or aphid transmission to indicator hosts is necessary in order to detect SMoV in commercial sources. Aphid transmission by *Chaetosiphon fragaefolii* is the favored method, with an acquisition access period of 4 to 6 h, and 2 to 3 h transmission access. In a mixed infection, this eliminates any persistently transmitted viruses (e.g., SCV and SMYEV). Semipersistently transmitted viruses (e.g., SVBV) can be distinguished easily by symptoms (12). SMoV symptoms appear 1 to 3 weeks postinoculation, depending on the severity of the virus strain.

The purpose of this work on SMoV was firstly, to improve its detection, and secondly, to obtain information on the genetic variation among isolates of this virus species.

MATERIALS AND METHODS

Plant material. All isolates, except Dutch isolates, were maintained in *Fragaria* spp. in a temperature and humidity controlled greenhouse at 20°C, 70% relative humidity, and a 16-h day length with supplemented artificial illumination when necessary. The Dutch isolates were sent to us as liquid nitrogen-frozen *F. vesca* leaf material stored on dry ice. Their long-term storage was at -80°C. The country of origin and severity of symptoms of each isolate tested are given in Table 1.

Total nucleic acid extraction. For reverse transcription-polymerase chain reaction (RT-PCR) detection and RT-PCR of the putative large coat protein (CPL) and putative RNA-dependent RNA polymerase (RdRp) regions, the same extraction method was employed. This incorporated an extra initial step in the silica capture protocol (21), whereby 0.1 g of fresh or frozen leaf material was ground in 1.0 ml SEB-buffer (0.14 M NaCl, 2 mM KCl, 2 mM KH₂PO₄, 8 mM Na₂HPO₄·2H₂O [pH 7.4], 0.05% vol/vol Tween 20, 2% wt/vol polyvinylpyrrolidone 40 [PVP 40], 0.2% wt/vol ovalbumin, 0.5% wt/vol bovine serum albumin, 0.05% wt/vol sodium azide) (27); 100 µl of the homogenate was then added to 400 µl of the silica capture grinding buffer (4.0 M guanidine thiocyanate, 0.2 M sodium acetate [pH 5.2], 25 mM EDTA, 1.0 M potassium acetate, and 2.5% wt/vol PVP 40). Fresh material was ground in a Bioreba (Reinach, Switzerland) bag with a hand-held bearing homogenator, and frozen tissue in a mortar and pestle. All 500 µl of the homogenate-buffer mix was then transferred to a 1.5 ml Eppendorf tube to which was added 100 µl of 10% N-lauryl sarkosyl and 5 µl of 2-mercaptoethanol. The mixture was incubated at 70°C with intermittent shaking for 10 min, placed on ice for 5 min, and then centrifuged at 13,000 × g for 10 min. Three hundred microliters of the supernatant fluid was then transferred to a new tube to which was added 150 µl of ethanol, 300 µl of 6 M sodium iodide, and 25 µl of resuspended silica, incubated at room temp for 10 min with intermittent shaking, and then centrifuged at 6,000 × g for 1 min. The pellet was resuspended in 500 µl of wash buffer (10 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 50.0 mM NaCl, 50% ethanol) and centrifuged at 6,000 × g for 1 min. The wash step was repeated once, and the pellet was allowed to dry for several minutes at room temperature before resuspending in 150 µl of sterile distilled water. The mix-

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ture was incubated at 70°C for 4 min, centrifuged at 13,000 × g for 3 min, and the supernatant fluid transferred to a new tube and stored at -20°C.

Detection of SMoV using RT-PCR.

For reverse transcription, 5 µl of total nucleic acid extract in 6.5 µl of sterile distilled water and 0.5 µl of sense primer (10 µM) were added; 4 µl of first strand buffer (5×) (Invitrogen, GmbH, Karlsruhe, Germany), 2 µl of DTT (0.1 M), and 1 µl dNTP's (10 mM) were added and incubated at 37°C for 10 min; 1 µl of MMLV or Superscript II (200 U/µl) (Invitrogen) was added and incubated at 42°C for 50 min. The enzyme was inactivated by incubating at 70°C for 10 min. PCR was done using Titanium *Taq* DNA Polymerase (Clontech, Palo Alto, CA) according to the manufacturer's instructions in a Robocycler (Stratagene, Heidelberg, Germany). Sense and antisense primers were, respectively, Smdetncr4a 5' TAAGCGACCACGACTGTGACAAAG 3' and Smdetncr1b 5' ATTCGGTTCACGTCCTAGTCTCAC 3'. For all primer pairs, cycling conditions consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and a final elongation step at 72°C for 5 min. PCR products were separated by electrophoresis in 1% agarose gels and visualized under UV light after staining with ethidium bromide.

RT-PCR using CPL, RdRp, and degenerate primers. Except for minor modifications, RT-PCR was carried out as above with the following primers. For partial amplification of the CPL region, sense and antisense primers were, respectively, Smpc1a 5' GGACCTACG-GATCTTGGAAGT 3' and Smpc1b 5' ACCCGCACAACCTTGTCGGAGG 3'. For partial amplification of the RdRp region, sense and antisense primers were, re-

spectively, Smpol1a 5' GCTTGTGCC-AAAGATGAGAAA 3' and Smpol1b 5' AGAAACAACGTTGTCTGCTCC 3'. For both the CPL and RdRp primers, lower annealing temperatures, minimum 44°C, were employed for some samples. Takara *La Taq* (Takara Shuzo Co., Shiga, Japan) was used with the reaction mixture recommended by the manufacturer. Degenerate primers for CPL were 5' GRATGACYACYAARGGTTC 3' and 5' AARAYNACRAACATCAT 3', based on the most conserved regions amplified by Smpol1a and Smpol1b in 11 isolates. Amplification conditions were the same as described above except that the annealing temperature was 42°C. PCR products were separated by electrophoresis in 1% agarose gels and visualized under UV light after staining with ethidium bromide.

Cloning, sequencing, and sequence analysis. PCR fragments were excised from the gel by GeneClean (Bio 101, Carlsbad, CA) and ligated into pBluescript KS with a T-overhang (2) at the *EcoRV* site. Competent *Escherichia coli* DH5α cells were then transformed (22) and plated on LB agar supplemented with ampicillin at 100 µg/ml and X-gal at 100 ng/ml. White colonies were then grown in a

selective medium, and plasmids were purified using the Concert rapid miniprep system (Invitrogen). Two independent clones from each of the sequences presented here were sequenced on both strands. To check for errors introduced by RT-PCR, a transcript generated from a 300-bp insert in pBluescript KS was reverse transcribed as described above and then amplified in 10 independent PCRs using Titanium *Taq* DNA polymerase. The generated fragments were then cloned, sequenced, and analyzed. All clones were sequenced with an ABI Prism Sequence Detection System (Applied Biosystems, Foster City, CA) and analyzed with the HUSAR computer program (German Cancer Research Center, Heidelberg, Germany). Multiple alignments were then done with the CLUSTAL X program (25) after bootstrapping in 1,000 replicates. Phylogenies were viewed by the NJplot program (16). Percent nucleotide and amino acid similarities between sequences were calculated using the HUSAR program.

RESULTS

Detection of SMoV isolates by RT-PCR. All 16 isolates (Table 1), tested by RT-PCR with primers Smdetncr4a and

Fig 1. 1% agarose gel separation of a 460-bp polymerase chain reaction (PCR) product amplified from various isolates of Strawberry mottle virus (SMoV) using primers Smdetncr4a and Smdetncr1b. Lanes 1 and 20, DNA 100-kb ladder (MBI Fermentas); lanes 2 to 17, SMoV isolates (see Table 1 for isolate description), respectively, 197/3a, 197/3b, 1509/4, 1248, 167, HD, ST, 1134, 1166, 1141, 1265, 1266, 1278, 1280, 9032.001M7, 3CH; lane 18, healthy *Fragaria vesca*; lane 19, water control. 4 µl from a 20-µl reaction was loaded for each sample.

Table 1. Isolates testing positive for Strawberry mottle virus (SMoV) using primers Smdetncr4a and Smdetncr1b

Isolate	Country ^a	Host ^b	Symptoms ^c	Year ^d	CPL ^e	POL ^e
197/3A	CZ	<i>F. ananassa</i>	–	1989	AJ496149	AJ496161
197/3B	CZ	UC4	+	1989	AJ496150	AJ496162
1509/4	PL	UC5	++	1999	AJ496147	AJ496159
1248	PL	UC10	++	1999	AJ496142	AJ496157
167	PL	UC10	+++	1999	AJ496148	AJ496160
HD	DE	UC4	+++	1975	AJ496153	NT
ST	DE	UC4	+	1975	AJ496154	NT
1134 ^e	NL	UC5	++	1987	AJ311876	AJ311875
1141	NL	UC5	+++	1987	AJ496140	NT
1166	NL	UC5	+	1987	AJ496141	AJ496156
1265	NL	UC5	+	1987	AJ496143	NT
1266	NL	UC5	+	1987	AJ496144	NT
1278	NL	UC5	++	1987	AJ496145	AJ496158
1280	NL	UC5	+	1987	AJ496146	NT
9032.001M7	US	<i>F. vesca</i>	++	1960s	AJ496152	NT
3CH	CH	<i>F. chiloensis</i>	–	2001	AJ496151	AJ496163

^a Country of origin: CZ, Czech Republic; PL, Poland; DE, Germany; NL, The Netherlands; US, United States of America; CH, Chile.

^b Host description: species or indicator plant (UC4, *Fragaria vesca* and *F. virginiana* hybrid; UC5, *F. vesca*, *F. chiloensis*, and *F. virginiana* hybrid; UC10, *F. virginiana*).

^c Degree of symptoms: –, symptomless; +, slight mottle; ++, severe mottle; +++, severe mottle with leaf deformation.

^d Year collected.

^e Accession numbers for each sequence of each isolate are included under respective headings CPL and POL. Accession numbers for isolate 1134 are for the complete sequence of RNA1 and 2 (26). NT, not tested.

Sm2ncr1b, were positive for the SMoV-specific product (Fig. 1). The PCR fragment was 460 bp long. The isolates had all previously been identified as SMoV by transmission to indicator plants (results not shown). Intensity of the fragments varied, although with even the faintest there could be no ambiguity as to the result. The less intense bands of the Dutch isolates can be most likely explained on the grounds that the materials were frozen before extraction. The PCR products of 11 out of the 16 isolates were sequenced. An alignment between all isolates and RNA1 and RNA2 of isolate 1134 was made (Fig 2), the latter two sequences being from previously obtained data (26). Four completely conserved regions of 20 or more bases (3 to 27, 56 to 78, 120 to 139, 154 to 190) were identified. There were no gaps in the alignment except at positions 346 to 354 where, for all isolates except 197/3A, a gap of one to five bases was present.

Variation in CPL and RdRp regions.

The primers used to partially amplify the CPL and RdRp regions were based on conserved regions within the genome. CPL and RdRp primers were located in RNA2 at 2092 to 2112 and 2553 to 2533, and in RNA1 at 4423 to 4444 and 5010 to 4990, respectively. For the CPL region, the conserved amino acid motifs LGS and PPT were identified by alignment of SMoV, with the related picorna-like viruses, SDV (Acc. no. D88459), Navel orange infectious mottling virus (NIMV) (Acc. no. AB000282), and Citrus mosaic virus (CiMV) (Acc. no. AB032751) (23). These motifs served as the template for the 3' end of the primers. For the RdRp region, the conserved amino acid motifs KxEK and GDD, designated motifs I and VI by Koonin (7), served similarly as templates for primer design. In alignments with other related viruses, these two motifs were the most conserved. Amplification of both

regions proved difficult, more often than not resulting in no product or the amplification of a spurious product, especially at lower annealing temperatures. RdRp primers successfully yielded fragments of the correct size in nine of the isolates, while CPL primers yielded products from 11 isolates: 197/3A, 197/3B, 1509/4, HD, ST, 1134, 1141, 1265, 1280, 9032.001M7, 3CH. The use of Takara La *Taq* DNA polymerase improved amplification of the desired products at lower annealing temperatures for certain isolates. Degenerate primers designed in the same region as the CPL and RdRp primers were no better at amplifying the expected fragments (results not shown). Degenerate primers were also designed on the most conserved regions of those extant sequences generated by the CPL and RdRp primers. For the CPL region, all remaining isolates were successfully amplified and cloned. For the RdRp region, amplification using these

Fig 2. Alignment of a ~396-nt stretch within the 3' noncoding region of 11 isolates of Strawberry mottle virus (SMoV) and RNA1 and 2 of isolate 1134. The consensus, which indicates bases common to all isolates, is shown at the top. Those bases, for each position, that are in a minority are boxed. Position of sequence within genome of isolate 1134 is for RNA1 6209-6602, and for RNA2 4792-5185. Accession numbers with isolate name in parentheses: AJ496581 (1141), AJ496582 (1166), AJ496583 (1248), AJ496584 (1265), AJ496585 (1266), AJ496586 (1278), AJ496587 (1280), AJ496588 (1509/4), AJ496589 (197/3A), AJ496590 (197/3B), AJ496591 (3CH).

degenerate primers was unsuccessful, with spurious amplification.

Sequence analysis of the putative CPL region (327 nt; 108 aa) showed nucleotide and amino acid identities ranged from 72.8 to 99.7% and 92.6 to 100%, respectively (Table 2). The lowest identities were with the two German isolates HD and ST, which formed a separate, although not statistically significant clade. All three Polish isolates (1509/4, 1248, 167) and four Dutch isolates (1134, 1141, 1265, and 1280) formed two separate clades (Fig. 3). Identities of the 546 nt of the putative RdRp gene of nine isolates ranged from 73.4 to 100%, and of the corresponding 182 aa sequence 94 to 100% (Table 3). All three Polish isolates tested were found to be identical. The phylogenetic relationships between isolates (Fig. 4) were similar to that for the CPL region. Amplification products of the NCR generated by the detection RT-PCR of 11 isolates were cloned and sequenced. Based on the sequence data provided by 10 clones (not shown) generated by RT-PCR from one transcribed clone, the percent RT-PCR error frequency [(number of base misincorporations/number of bases analyzed) × 100] was calculated to be 0.12%. Such an error rate did not affect the relationships determined among isolates.

DISCUSSION

The RT-PCR method for detection of SMoV in *Fragaria* spp. as described here has a clear advantage over the present indexing methods, enabling a faster, more reliable indexing of strawberry material. The choice of which region of the viral genome to use was based on two observations: first, that it had already been shown for the nepovirus *Cherry leaf roll virus* (CLRv) (1) that there was a relatively high degree of conservation in the 3' noncoding region (3'NCR) between isolates, and second, that the degree of homology between the 3'NCRs of RNA1 and 2 for SMoV

isolate 1134 was also high (95.7%), in theory providing a higher template molarity than for any other region within the genome. Based on the alignment of RNA1 and 2 of SMoV isolate 1134, various primers were designed and tested, and finally the pair used in this study was selected.

One of the main obstacles to overcome in this work was that of finding a suitable

nucleic acid extraction method for *Fragaria* spp. material, which along with other berry plants is known to be particularly recalcitrant. Secondary metabolites, such as tannins, polyphenols, and polysaccharides, reportedly inhibit *Taq* polymerase activity (17). Among the methods reported (10,14,17,23), the cheapest, fastest, and most reliable method was the adapted sil-

Fig 3. Phylogenetic tree based on the alignment from various isolates of a 546-nt stretch within the putative RNA-dependent RNA polymerase gene of Strawberry mottle virus (SMoV). Description of the isolates is shown in Table 1. Bootstrap values for 1,000 replicates are given at each node. The scale bar represents the number of substitutions for each base. Satsuma dwarf virus (SDV) (Acc. No. AB009958) was used as the outgroup.

Table 2. Percent nucleotide (above diagonal) and amino acid (below diagonal) identities between 16 isolates of Strawberry mottle virus (SMoV) based on a 327 nt/108 aa region of the putative large coat protein gene (CPL)

	197/3A	197/3B	1509/4	1248	167	HD	ST	1134	1141	1166	1265	1266	1278	1280	9032.001M7	3CH
197/3A	—	83.2	87.8	86.5	85.3	79.8	77.4	86.9	86.2	83.5	87.8	83.5	86.2	87.8	80.7	85.0
197/3B	97.2	—	83.2	83.2	82.0	72.8	77.7	85.9	85.9	82.0	85.9	80.7	82.6	85.6	79.8	86.5
1509/4	97.2	96.3	—	98.5	97.2	75.8	75.8	92.4	92.0	84.1	92.7	83.8	94.8	91.7	81.0	87.2
1248	97.2	96.3	100	—	98.8	75.5	76.1	93.0	92.7	84.4	93.3	83.2	94.2	92.4	80.7	87.8
167	96.3	95.4	99.1	99.1	—	75.2	77.4	91.7	91.4	84.1	92.0	82.0	93.3	91.1	82.0	86.5
HD	91.7	92.6	90.7	90.7	91.7	—	82.3	75.8	76.5	74.9	75.2	76.5	76.1	74.9	74.9	76.1
ST	94.4	93.5	91.7	91.7	92.6	96.3	—	78.3	78.9	78.3	78.3	77.7	75.2	78.9	78.0	74.3
1134	96.3	97.2	99.1	99.1	98.1	91.7	92.6	—	99.7	83.5	99.1	82.3	92.0	97.9	80.4	86.2
1141	96.3	97.2	99.1	99.1	98.1	91.7	92.6	100	—	84.1	98.8	82.9	91.7	97.6	81.0	85.6
1166	94.4	93.5	93.5	93.5	94.4	91.7	91.7	92.6	92.6	—	83.5	92.7	83.2	82.9	93.3	81.7
1265	96.3	97.2	99.1	99.1	98.1	91.7	92.6	100	100	92.6	—	82.3	91.7	98.8	80.4	85.9
1266	95.4	94.4	94.4	94.4	93.5	90.7	90.7	93.5	93.5	99.1	93.5	—	82.9	82.6	92.4	81.7
1278	95.4	94.4	98.1	98.1	98.1	89.8	90.7	97.2	97.2	92.6	97.2	92.6	—	90.5	82.0	87.2
1280	96.3	97.2	99.1	99.1	98.1	91.7	92.6	100	100	92.6	100	93.5	97.2	—	80.4	85.3
9032.001M7	91.7	92.6	90.7	90.7	91.7	90.7	90.7	91.7	91.7	97.2	91.7	96.3	89.8	91.7	—	78.6
3CH	99.1	98.1	98.1	98.1	97.2	92.6	93.5	97.2	97.2	95.4	97.2	96.3	96.3	97.2	92.6	—

ica capture method described here. The silica capture method described by Rott and Jelkmann (21), from which the method here was adapted, was not adequate in that subsequent RT-PCRs were not consistent. Inconsistency in PCR detection of viruses in *Fragaria* spp. has also been reported for SCV (18) and SVBV (13). Nevertheless, by a 1 in 6 dilution of the sample homogenate in SEB prior to nucleic acid extraction, a robust and reliable detection method was provided (M. Klerks, *personal communication*), as confirmed by the effectiveness of primers Smdetncr4a and Sm2ncr1b. This can be simply explained on the basis that viral RNA is still at a sufficiently high concentration to be detected, and that the inhibitory effects of other compounds present in the extraction are sufficiently reduced so as to become negligible. Other dilutions of the sample homogenate were tested, but the dilution used here appeared optimal for the primers used.

The initial amplification by RT-PCR of the RdRp and CPL regions using primers designed around conserved motifs was successful only for certain isolates. The most likely explanation for this is that nucleotide sequence variation was such that the primers were unable to anneal efficiently. This was further supported by the fact that those isolates that failed to produce a product with the RdRp primers were for German isolates HD and ST, the most divergent genetically, as indicated by the phylogram for the CPL region (Fig. 3). The U.S. isolate may also have evaded PCR amplification for the same reason. The use of degenerate primers based on the most conserved regions in the CPL region enabled the remaining isolates to be amplified and analyzed. The degree of degeneracy in the CPL-specific primers used here was greater than that for a degenerate pair of primers designed for the RdRp region, although curiously the latter pair was not effective at yielding a specific product (results not shown).

Sequence alignment of the amplified products generated using the detection provided information on the level of homology between isolates, and serves as a template for further refinements in future detection methods. The alignment shown in Figure 2 cannot be used in any analyses,

as it is unclear, except for isolate 1134, from which RNA species the fragment is derived.

In analyzing the CPL and RdRp partial sequences, it is important not to lose sight of the fact that together they only represent approximately 7% of the complete genome, although that is not to say that the results are not representative and cannot be extrapolated to provide information on phylogenies. Indeed, although for seven isolates no sequence data were obtained on the RdRp region, the fact that the phylograms for both regions of the genome somewhat resemble each other provides additional evidence for the above assumption. In terms of the grouping within the phylograms for the CPL and RdRp regions (Figs. 3 and 4), there was a clear indication that the isolates grouped according to their geographical origin. This was certainly true for the Polish isolates, which were

identical at a nucleic acid level in the RdRp region. The Dutch isolates also had a tendency to group together with some divergent isolates for the CPL region. Interestingly, the two New World isolates (3CH and 9032.001M7), although probably in no way sympatric, were grouped separately with other European isolates; the Chilean isolate formed a single branch in both phylograms. Given the geographical correlation with phylogenies, the two Czech isolates (197/3A and 197/3B) were especially divergent considering they may have originally come from the same source, although their exact history is uncertain. The resulting divergence in this case may be due to either contamination from a distinct source or adaptation to a different host over prolonged isolation, possibly as a result of either quasi-species-like characteristics, as identified for the nepovirus *Grape fanleaf virus* (15), or genetic drift. In comparison

Fig 4. Phylogenetic tree based on the alignment from various isolates of a 327-nt stretch within the putative large coat protein gene of *Strawberry mottle virus* (SMoV). Description of the isolates is shown in Table 1. Bootstrap values for 1,000 replicates are given at each node. The scale bar represents the number of substitutions for each base. Satsuma dwarf virus (SDV) (Acc. No. AB009959) was used as the outgroup.

Table 3. Percent nucleotide (above diagonal) and amino acid (below diagonal) identities between nine isolates of *Strawberry mottle virus* (SMoV) based on a 546 nt/182 aa region of the putative RNA-dependent RNA polymerase

	197/3A	197/3B	1509/4	1248	167	1134	1166	1278	3CH
197/3A	—	73.4	76.6	76.6	76.6	75.1	75.3	74.5	76.7
197/3B	94.0	—	77.8	77.8	77.8	76.2	76.4	76.2	79.5
1509/4	94.0	95.1	—	100.0	100.0	77.1	77.3	76.7	84.6
1248	94.0	95.1	100.0	—	100.0	77.1	77.3	76.7	84.6
167	94.0	95.1	100.0	100.0	—	77.1	77.3	76.7	84.6
1134	94.5	95.1	95.1	95.1	95.1	—	99.8	99.3	78.2
1166	95.1	95.6	95.6	95.6	95.6	99.5	—	99.5	78.4
1278	94.0	94.5	95.1	95.1	95.1	98.4	98.9	—	77.7
3CH	94.0	96.2	98.9	98.9	98.9	95.1	95.6	94.5	—

with the SDV group of viruses, in which SMOV is tentatively classified, the percent amino acid identities found for the complete CPL are lower, ranging from 82 to 98% (4). It may well be the case that for SMOV, the homology between isolates outside the region sequenced here is significantly lower. A CP amino acid motif of high homology shared with more distantly related viruses, including several picornaviruses and sequiviruses, and found consistently in all SMOV isolates was GxUxUxxxPxG, where x represents any base and U represents the bulky aliphatic residues (I, L, M, V) (5). Similarly, the RdRp motifs of positive-strand RNA viruses V (SGxxxTxxxNSUU) and IV (DxTxXD) were also present (7).

In summary, this work has developed an RT-PCR detection method for SMOV and provides sequence information that should lead to yet further improvements in detection. It also firmly associates SMOV with the disease and gives an insight into the degree of variation and the genetic relationships between isolates.

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In the article "The Detection and Variation of Strawberry mottle virus" by J. R. Thompson and W. Jelkmann, pages 385-390, the captions for Figures 3 and 4 were reversed. They should appear as follows.

Fig 3. Phylogenetic tree based on the alignment from various isolates of a 327-nt stretch within the putative large coat protein gene of Strawberry mottle virus (SMoV). Description of the isolates is shown in Table 1. Bootstrap values for 1,000 replicates are given at each node. The scale bar represents the number of substitutions for each base. Satsuma dwarf virus (SDV) (Acc. No. AB009959) was used as the outgroup.

Fig 4. Phylogenetic tree based on the alignment from various isolates of a 546-nt stretch within the putative RNA-dependent RNA polymerase gene of Strawberry mottle virus (SMoV). Description of the isolates is shown in Table 1. Bootstrap values for 1,000 replicates are given at each node. The scale bar represents the number of substitutions for each base. Satsuma dwarf virus (SDV) (Acc. No. AB009958) was used as the outgroup.