Genetic Diversity of Nine Non-Recombinant Potato virus Y Isolates From Three Biological Strain Groups: Historical and Geographical Insights

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Abstract

Potato virus Y (PVY) isolates from potato currently exist as a complex of six biologically defined strain groups all containing nonrecombinant isolates and at least 14 recombinant minor phylogroups. Recent studies on eight historical UK potato PVY isolates preserved since 1984 found only nonrecombinants. Here, four of five PVY isolates from cultivated potato or wild *Solanum* spp. collected recently in Australia, Mexico, and the U.S.A. were typed by inoculation to tobacco plants and/or serological testing using monoclonal antibodies. Next, these five modern isolates and four additional historical UK isolates belonging to biological strain groups PVY^C, PVY^Z, or PVY^N obtained from cultivated potato in 1943 to 1984 were sequenced. None of the nine complete PVY genomes obtained were recombinants. Phylogenetic analysis revealed that the four historical UK isolates were in minor phylogroups PVY^{C1} (Y^C-R), PVY^{O-O} (Y^Z-CM1), PVY^{NA-N} (Y^N-M),

or PVY^{Eu-N} (Y^N-RM), Australian isolate Y^O-BL2 was in minor phylogroup PVY^{O-O5}, and both Mexican isolate Y^N-Mex43 and U.S.A. isolates Y^N-MT12_Oth288, Y^N-MT12_Oth295, and Y^N-WWAA150131G42 were in minor phylogroup PVY^{Eu-N}. When combined, these new findings and those from the eight historical UK isolates sequenced earlier provide important historical insights concerning the diversity of early PVY populations in Europe and the appearance of recombinants in that part of the world. They and four recent Australian isolates sequenced earlier also provide geographical insights about the geographical distribution and diversity of PVY populations in Australia and North America.

Keywords: potato virus Y, pathogen diversity, potato, historical isolates, biological strain groups, phylogenetics

Potato (*Solanum tuberosum*) is one of the world's most important food crops (FAOSTAT 2017), and potato virus Y (PVY; family *Potyviridae*, genus *Potyvirus*) currently causes the most important virus disease of potato worldwide. It gives rise to substantial losses in potato tuber yield, reduces tuber quality by causing potato tuber necrotic ringspot disease (PTNRD) in PVY-sensitive cultivars, and disrupts healthy seed potato production (Gray et al. 2010; Jones 2014; Karasev and Gray 2013a; Kreuze et al. 2020; Singh et al. 2008; Valkonen 2007). It has a single stranded, positive sense RNA genome of ~9.7 kb, is nonpersistently aphid-transmitted (Karasev and Gray 2013a; Kerlan 2006), and is also contact-transmissible (Coutts and Jones 2015). In addition, PVY causes economically important diseases in other solanaceous crops, especially tobacco, tomato, and pepper (Blanchard

et al. 2008; Green et al. 2017b; Kehoe and Jones 2016; Kerlan 2006; Kerlan and Moury 2008; Moury 2010; Quenouille et al. 2013).

Biological strain groups consisting of PVY isolates from potato are distinguished by whether they elicit hypersensitive responses (HR) in potato cultivar differentials carrying hypersensitivity genes or systemic vein necrosis symptoms in tobacco. Strain groups PVY^C, PVY^O, and PVY^Z elicit HR phenotypes with hypersensitivity genes *Nc*, *Ny*, or *Nz*, respectively. Strain groups PVY^N and PVY^E overcome all three, but differ in the phenotypes they induce in tobacco, with only PVY^N eliciting vein necrosis (Chikh-Ali et al. 2014; Cockerham 1970; Jones 1990; Karasev and Gray 2013a, b; Kehoe and Jones 2016; Kerlan et al. 2011; Rowley et al. 2015; Singh et al. 2008). Previously, biological strain groups PVY^C and PVY^O were thought to coincide with major phylogroups PVY^C and PVY^O, respectively (Boonham et al. 2002; Moury et al. 2002). However, this proved incorrect as isolates within biological strain groups PVY^Z and new strain group PVY^D fitted within major phylogroups PVY^O and PVY^C, respectively (Kehoe and Jones 2016). As the number of complete PVY genome sequences from different world regions grows, phylogenetic nomenclature based on biological, geographical, and sequence names is becoming increasingly unsustainable so substituting Latinized numerals for current phylogroup names was suggested (Jones 2014; Jones and Kehoe 2016; Kehoe and Jones 2016). When Gibbs et al. (2017) examined the phylogenetics of the global population of PVY isolates with complete genomes, 42% were nonrecombinants and 58% recombinants. The nonrecombinants were all within major phylogroups PVY^C, PVY^N, or PVY^O, while recombinants between PVY^N and PVY^O were in major phylogroups R-1 or R-2. The principal parents of R-1 and R-2 were PVY^N or PVY^O, respectively, and vice versa for their secondary parents. The minor phylogroups within these major phylogroups [numerals from PVY classification

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*The e-Xtra logo stands for "electronic extra" and indicates that one supplementary table is published online.

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Table 1. Isolates of potato virus Y sequenced in this study, or by the authors listed

Isolate	Biological strain group	Phylogroup (this study or Kehoe and Jones 2016)	Original host	Year isolated	Geographical origin	First isolate reference	Strain group reference	CP accession no. (Kehoe and Jones 2011)	Genome accession no. from this study or Kehoe and Jones (2016)
Historical isolates sequenced in this study									
R	Y ^C	Y ^{C1}	Potato cv. Edgecote Purple	1943	United Kingdom	Bawden and Kassanis (1947)	Jones (1990)	JN034578	MT200665
RM	Y ^N	Y ^{NA-N}	Potato cv. Record	1981	Terrington, England	Jones (1990)	Jones (1990)	-	MT200666
M	Y ^N	Y ^{Eu-N}	Potato cv. unknown	<1983	Unknown	Jones (1990)	Jones (1990)	-	MT200667
CM1	Y ^Z	Y ^{O-O}	Potato cv. Cara	1981	Cambridge, England ^a	Jones (1990)	Jones (1990)	-	MT200668
Recent isolates sequenced in this study									
BL2	-	Y ^{O-O5}	Potato cv. Bliss	2014	Pemberton, Western Australia	This study	-	-	MT200669
Mex43	-	Y ^{Eu-N}	Wild <i>Solanum</i> sp.	2014	Jalisco, Mexico	This study	-	-	MT200670
MT12_Oth288	-	Y ^{Eu-N}	Potato cv. Ranger Russet	2012	Washington, U.S.A.	This study	-	-	MT200671
MT12_Oth295	-	Y ^{Eu-N}	Potato cv. Ranger Russet	2012	Washington, U.S.A.	This study	-	-	MT200672
WWA150131_G4_8	-	Y ^{Eu-N}	Potato cv. Chieftan	2015	Washington, U.S.A.	This study	-	-	MT200673
Historical isolates already with complete genome sequences									
Y ^C -CT	Y ^C	Y ^{C2}	Potato cv. Catriona	<1980	United Kingdom	Jones (1990)	Jones (1990)	JN034577	KP691324
Y ^C -CRM1	Y ^C	Y ^{C2}	Potato cv. Pentland Crown	1982	Cambridge, England ^a	Jones (1990)	Jones (1990)	JN034576	KP691321
Y ^O -CRN	Y ^O	Y ^{O-O}	Potato cv. Pentland Crown	1982	Cambridge, England ^a	Jones (1990)	Jones (1990)	JN034573	KP691323
Y ^O -KE	Y ^O	Y ^{O-O}	Potato cv. King Edward	1943	United Kingdom	Bawden and Kassanis (1947)	Jones (1990)	JN034571	KP691327
Y ^O -RS	Y ^O	Y ^{O-O}	Potato cv. Record	1981	Terrington, England	Jones (1990)	Jones (1990)	JN034574	KP691330
Y ^Z -CM2	Y ^Z	Y ^{O-O}	Potato cv. Cara	1984	Cambridge, England ^a	Jones (1990)	Jones (1990)	JN034572	KP691319
Y ^Z -CRM2	Y ^Z	Y ^{O-O}	Potato cv. Pentland Crown	1982	Cambridge, England ^a	Jones (1990)	Jones (1990)	JN034568	KP691322
Y ^Z -DS	Y ^Z	Y ^{O-O}	Potato cv. Desiree	1984	Cambridge, England	Jones (1990)	Jones (1990)	JN034569	KP691326
More contemporary isolates already with complete genome sequences									
CN1	-	Y ^{C1}	Tomato cv. unknown	2006	Carnarvon, Western Australia	Kehoe and Jones (2011)	Kehoe and Jones (2016)	JN034579	KP691320
KIP1 ^b	Y ^D	Y ^{C2}	Potato cv. Kipfler	2008	Queensland, Australia ^b	Kehoe and Jones (2016)	Kehoe and Jones (2016)	-	KP691329
BL	Y ^O	Y ^{O-O5}	Potato cv. Bliss	2003	Busselton, Western Australia	Kehoe and Jones (2011)	Kehoe and Jones (2016)	JN034575	KP691318
DEL3	Y ^O	Y ^{O-O5}	Potato cv. Delaware	2011	Manjimup, Western Australia	Kehoe and Jones (2011)	Kehoe and Jones (2016)	JN034566	KP691325

(Continued on next page)

^a From National Institute of Agricultural Botany plots, Cambridge, England.^b Isolate from shop purchased potato tubers originally from Queensland, Australia.

Table 1. (Continued from previous page)

Isolate	Biological strain group	Phylogroup (this study or Kehoe and Jones 2016)	Original host	Year isolated	Geographical origin	First isolate reference	Strain group reference	CP accession no. (Kehoe and Jones 2011)	Genome accession no. from this study or Kehoe and Jones (2016)
ATL1	Y ^Z	Y ^{O-O5}	Potato cv. Atlantic	2012	Manjimup, Western Australia	Kehoe and Jones (2016)	Kehoe and Jones (2016)	-	KP691317
NE38	-	Y ^{O-O}	Potato cv. unknown	2004	Nebraska, U.S.A.	Green et al. (2017a)	Green et al. (2017a)	-	KY848004
ME_236_120	-	Y ^{O-O}	Potato cv. unknown	2004	Maine, U.S.A.	Green et al. (2017a)	Green et al. (2017a)	-	KY847962
NY100003	-	Y ^{O-O}	Potato cv. unknown	2010	New York, U.S.A.	Green et al. (2017a)	Green et al. (2017a)	-	KY848012
SASA-110	-	Y ^{O-O}	Potato cv. unknown	2004	United Kingdom	Barker et al. (2009)	Barker et al. (2009)	-	AJ585195
O-139	Y ^O	Y ^{O-O5}	Potato cv. unknown	1993	Eastern Canada	Singh and Singh (1996)	Singh and Singh (1996)	-	U09509
ME120	-	Y ^{O-O}	Potato cv. unknown	2005	Maine, U.S.A.	Karasev et al. (2011)	Karasev et al. (2011)	-	HQ912892
ME236_4	-	Y ^{O-O}	Potato cv. unknown	2004	Maine, U.S.A.	Karasev et al. (2011)	Karasev et al. (2011)	-	HQ912891

system of Kehoe and Jones (2016) in parentheses] were: C with C1(II) and C2(III); O with O (= I) and O5 (= X); N with Eu-N (= IV), XIII, and NA-N (= IX); R-1 with SYR-I (XII), NTN-B (VI), SYR-II (XI), N-Wi (VII), and N:O (VIII); and R-2 with NTN-A (V) (Gibbs et al. 2017).

Kehoe and Jones (2016) compared the biological and complete genome properties of eight historical UK (1943 to 1984) and five Australian (2003 to 2012) PVY isolates, none of which were recombinants. The UK isolates belonged to biological strain groups PVY^C, PVY^O, or PVY^Z (Jones 1990), and the Australian isolates to PVY^O, PVY^Z, or new strain group PVY^D, which elicited putative hypersensitivity gene *Nd*; the tomato isolates were left ungrouped as they proved poorly adapted to infect potato systemically (Kehoe and Jones 2016). Putative gene *Nd* was present in all but three of 39 Australasian, European, or North American potato cultivars (Jones and Vincent 2018). On phylogenetic analysis of complete genomes, the Australian PVY isolates from potato were in minor phylogroups Y^{O-O}, Y^{O-O5}, or Y^{C2}, the Australian tomato isolate sequenced was in minor phylogroup Y^{C1}, and the historical UK potato isolates were in Y^{O-O} or Y^{C2}. Moreover, biologically defined PVY^O and PVY^Z isolates were within Y^{O-O} or Y^{O-O5}, PVY^C isolates within Y^{C2}, PVY^D within Y^{C2}, and the tomato isolate in Y^{C1}. In the present study, whole genomes were obtained of four additional historical UK isolates from strain groups PVY^C, PVY^N, or PVY^Z previously studied by Jones (1990). They were compared with (i) the eight historical UK and four recent Australian PVY isolates sequenced by Kehoe and Jones (2016), (ii) five modern non-recombinant PVY isolates collected in Australia, Mexico, or the United States, and (iii) other nonrecombinant isolates from GenBank.

Four PVY isolates were obtained from freeze dried specimens kept in Perth, Western Australia since importation from England in 1986 under an Australian quarantine permit (Table 1). They had been freeze-dried in sealed glass ampoules from fresh infected potato or tobacco leaf tissue in 1984 and never revived thereafter. Isolate Y^{C-R} was the classical Rothamsted PVY^C isolate obtained in 1943 from cv. Edgecotte Purple (Bawden and Kassanis 1946, 1947; Kassanis and Govier 1971). Isolates Y^{N-RM} and Y^{Z-CM1} were obtained in 1981 from cv. Record growing at Terrington, Norfolk, England, or cv. Cara growing at Cambridge, England, respectively. Isolate Y^{N-M}

was from the UK in the early 1980s (cultivar and location not recorded). Before freeze drying in England, these four isolates were maintained by subculture in tobacco plants (cv. White Burley) or replanting infected potato tubers (Jones 1990). Their biological properties were reported previously (Jones 1990). In October 2015, single glass ampoules containing each historical PVY isolate were broken in Australia where dry leaf tissue from each of them (approximately 0.5 g) was transferred into a sterile mortar for homogenization with 0.5 ml 0.1 M phosphate-buffered saline (pH 7.2). New isolate BL2 obtained in 2014 from potato cv. Bliss growing at Pemberton, Western Australia, during routine seed potato testing by ELISA was maintained thereafter in tobacco cv. White Burley. It was extracted from a fresh infected tobacco leaf in the same way. With each isolate, the resulting homogenate was applied to PlantSaver FTA Cards (Whatman), followed by drying and sealing in plastic bags. The five FTA cards were transported to the University of Idaho (UI), U.S.A., for further characterization. Isolate Mex43, collected during a survey of seed potato lots in 2014 at Tapalpa, Jalisco, Mexico, came from an asymptomatic wild *Solanum* spp. weed growing in a service road alongside a potato field. The sample, which tested positive for PVY using an immuno-strip test (Agdia, Elkhart, IN), was pressed onto a PlantSaver FTA Card (Whatman) and sent to UI as described above. Isolates MT12_Oth288 and MT12_Oth295 were collected in 2012 from infected cv. Ranger Russet plants growing in two commercial potato seed lots planted with seed tubers originally from Montana and being grown at Othello, Washington State (WA). Isolate WWA150131_G4_8 was collected in 2015 from a cv. Chieftain plant growing in a winter grow-out test in western WA, U.S.A. After initial typing using ELISA (Nikolaeva et al. 2012) and RT-PCR (Chikh-Ali et al. 2013; Lorenzen et al. 2006), these three isolates were cultured in tobacco cv. White Burley. Several reference PVY isolates from the UI collection belonging to nonrecombinant major phylogroup PVY^C, and minor phylogroups PVY^{O-O}, PVY^{O-O5}, PVY^{Eu-N}, and PVY^{NA-N} were used for comparisons as described previously (Chikh-Ali et al. 2013; Funke et al. 2017; Green et al. 2017b; Nikolaeva et al. 2012).

RNA was extracted from FTA cards as described by Ndunguru et al. (2005). The resulting RNA pellets were dissolved in 30 µl nuclease-free water, heated at 65°C for 5 min, and then chilled on ice for 3 min. Total nucleic acids were extracted from infected potato

and tobacco leaves as described previously (Dellaporta et al. 1983; Green et al. 2017a; Hu et al. 2009). They were typed using two different multiplex RT-PCR assays as described by Lorenzen et al. (2006) and Chikh Ali et al. (2010). Reverse transcription and PCR reactions were as described previously (Chikh-Ali et al. 2013; Green et al. 2017a). Control PVY isolates from the UI laboratory collection were used, and the assays on infected samples involved immunocapture RT-PCR (Chikh-Ali et al. 2013).

Serological reactivity of WWA150131_G4_8, MT12_Oth288, and MT12_Oth295 was tested by TAS-ELISA (Nikolaeva et al. 2012); all such tests included control PVY isolates with serological patterns characteristic of PVY^{O-O}, PVY^N, and PVY^{O-O5}. A polyclonal PVY antiserum and four phylogroup specific monoclonal antibodies were used: SASA-O recognizing PVY^{O-O}, PVY^{O-O5}, PVY^{N-Wi/N:O}, and PVY^C, and SASA-N recognizing PVY^N and PVY^{NTN} (SASA, Scottish Agriculture Science Agency, Edinburgh, Scotland); and 1F5 (Agdia, Elkhart, IN) recognizing PVY^N, PVY^{O-O5}, and PVY^{NTN} (Funke et al. 2017; Karasev et al. 2010).

Nearly whole genome sequences were obtained by sequencing overlapping RT-PCR products as described by Green et al. (2017a); due to the sequencing methodology used, ~100 nt of the terminal 5' end remained unsequenced. The sequencing strategy of Green et al. (2017a) involved direct sequencing of RT-PCR fragments generated by 48 primer pairs that amplified nearly the whole PVY genome. All PCR products were purified using the ExoSAP-IT for PCR Product Clean-Up (USB Products; Affymetrix Inc.), mixed with appropriate primers and submitted to Genewiz, Inc. (South Plainfield, NJ) for Sanger sequencing. These PCR products provided good sequence overlap, resulting in at least fourfold coverage of each PVY genome that represented a consensus; no genetic heterogeneity was present in the samples. The contigs obtained were assembled using the SeqMan Pro program of the Lasergene 9 Suite (DNASTAR). For multiple sequence alignments, MUSCLE (Edgar 2004) implemented in MEGA version 7 (Tamura et al. 2013) was used with default parameters. The alignment consisted of all nine newly sequenced isolates listed in Table 1. Sequence identity was checked using BLAST provided by the National Center for Biotechnology Information (NCBI) (Altschul et al. 1990). The aligned sequences were checked for recombination, using RDP (Martin and Rybicki 2000), GENECONV (Sawyer 1999), BOOTSCAN (Salminen et al. 1995), MAXCHI (Maynard-Smith 1992), CHIMAERA (Posada and Crandall 2001), 3Seq (Boni et al. 2007), and SiScan (Gibbs et al. 2000) methods in RDP4 v4.61 software (Martin et al. 2005). This recombination analysis was conducted as described by Green et al. (2017a), with an isolate considered recombinant if all six programs of the RDP package (RDP, GENCONV, Chimaera, MaxChi, Bootscan, SiScan) had significant support ($P < 0.0001$). Phylogeny inference based on whole genomes was by the maximum likelihood (ML) method implemented in MEGA 7. Bidens mosaic virus sequence (KF649336; Sanches et al. 2014) was used as an outgroup. Phylogroup diversity within and between nonrecombinant isolates was calculated using MEGA 7 (Tamura et al. 2013).

Prior to freeze-drying, historical UK isolates Y^N-M, Y^N-RM, Y^C-R, and Y^Z-CM1 had all been inoculated to plants of PVY differential potato cultivars and tobacco which established their biological strain groups (Jones 1990). When inoculated to tobacco plants, new isolates WWA150131_G4_8, MT12_Oth288, and MT12_Oth295 all caused systemic veinal necrosis typical of strain group PVY^N, but isolate BL2 caused a systemic mosaic so was not PVY^N (Table 2). Due to regulatory restrictions for introduction of pathogens to the U.S.A., isolate Mex43 could not be characterized biologically from FTA cards.

Based on ELISA results, the three PVY isolates from the U.S.A. had N-serotype, and all three were typed as PVY^{Eu-N} based on RT-PCR (Table 2). Isolates Mex43 and Y^N-M analyzed from FTA cards produced two characteristic bands, 328 and 398 bp, in the RT-PCR multiplex assay of Lorenzen et al. (2006), and three bands, 398, 633, and 1,307 bp, in the RT-PCR multiplex assay of Chikh-Ali et al. (2010). Therefore, they also belonged to PVY^{Eu-N}, as did control isolate N605. By contrast, isolate Y^N-RM produced a single 328-bp band in the Lorenzen assay and a single 1,307-bp band in the Chikh-Ali assay, so it belonged to PVY^{NA-N}, as did control isolate RRA1. Isolates Y^Z-CM1 and BL2 produced two characteristic bands, 267 and 689 bp, in the Lorenzen assay, and 532 and 853 bp bands in the Chikh-Ali assay, so belonged to PVY^O, like control isolate Oz. Isolate Y^C-R produced no bands in either Lorenzen or Chikh-Ali RT-PCR assays, resembling PVY^C isolates (Chikh-Ali et al. 2016; Green et al. 2017b).

Nearly complete, ca. 9.6 kb genomes of all nine isolates were deposited in the GenBank database under accession numbers MT200665 to MT200673 (Table 1). A single, 3,061-aa polypeptide was encoded by a single open reading frame (ORF) in each of their sequences. A short ORF called PIPO (Chung et al. 2008) was also present within their P3 cistrons. Sequence identity levels differed widely between the nine isolates, ranging from 99% between Mex43 and Y^N-M to 84% between Y^C-R and Y^N-M.

In phylogenetic reconstructions with the nine new sequences, and 56 sequences from nonrecombinants from the GenBank database representing all nonrecombinant PVY phylogroups available at that time, the newly sequenced isolates belonged to major phylogroups PVY^C, PVY^O, or PVY^N (Fig. 1). Isolate Y^C-R from 1943 was in minor phylogroup PVY^{C1} next to historical potato isolate Y^C-PRI-509 collected in 1938 in the Netherlands (Dulleman et al. 2011). Isolate Y^Z-CM1 was in minor phylogroup PVY^{O-O} close to isolate Y^Z-CM2 from the same potato cultivar, and in the same minor phylogroup as more contemporary isolate SASA-110 described in 2004 from Scotland (AJ585195; Barker et al. 2009). Isolate Y^N-M was placed in minor phylogroup PVY^{Eu-N}, close to modern PVY^{Eu-N} isolate N605 from Switzerland, but separately from other PVY^{Eu-N} isolates from outside Europe. Isolate Y^N-RM, however, was in minor phylogroup PVY^{NA-N}, close to modern PVY^{NA-N} isolates from North America and Japan. Modern Australian isolate BL2 was in minor phylogroup PVY^{O-O5}, close to several modern isolates from Australia and North America, but distant from BL, another Australian isolate from the same cultivar, which was in PVY^{O-O}. The four modern PVY^N isolates were all

Table 2. Summary of the biological, serological, and genetic typing data for the historical and contemporary isolates of potato virus Y characterized in this study

Isolate	Tobacco reaction ^a	Serotype ^b	Multiplex RT-PCR ^c		Genotype, final
			(1)	(2)	
PVY ^C _R	M	-	none	none	Y ^{C1}
PVY ^O _CM1	M	-	267, 689	532, 853	Y ^{O-O}
BL2	M	-	267, 689	532, 853	Y ^{O-O5}
PVY ^N _RM	VN	-	328	1,307	Y ^{NA-N}
WWA150131_G4_8	VN	N	328, 398	398, 633, 1,307	Y ^{Eu-N}
Mex43	-	-	328, 398	398, 633, 1,307	Y ^{Eu-N}
PVY ^N _M	VN	-	328, 398	398, 633, 1,307	Y ^{Eu-N}
MT12_Oth288	VN	N	328, 398	398, 633, 1,307	Y ^{Eu-N}
MT12_Oth295	VN	N	328, 398	398, 633, 1,307	Y ^{Eu-N}

^a M = mosaic and vein clearing; VN = vein necrosis; - = not tested.

^b N designates "N" serotype according to Nikolaeva et al. (2012).

^c RT-PCR typing was conducted with two primer sets, according to (1) Lorenzen et al. (2006) and (2) Chikh-Ali et al. (2010), numbers are characteristic PCR bands in base pairs (bp); none = no bands produced.

Fig. 1. Condensed, maximum-likelihood phylogenetic tree for the selected full genomes of nonrecombinant potato virus Y (PVY) isolates. Bold red color (lighter gray in print) indicates the “old” (pre-1985), and bold blue color (darker gray in print) indicates “modern” PVY sequences from this study. All other sequences are from the GenBank database. Sequence alignment was done using ClustalW provided in MEGA 7, and the best model was selected by MEGA 7, GTR+I+G (12 Gamma categories). Bootstrap values higher than 70% are shown at the corresponding nodes. Abbreviations of the PVY isolate names are followed by the corresponding GenBank numbers. Brackets on the right side indicate major phylogroups PVY^O, PVY^C, PVY^N, and Chile 3, and the minor phylogroups within them. Bidens mosaic virus sequence used as outgroup.

in minor phylogroup PVY^{Eu-N} (Fig. 1). The two modern isolates originally from Montana grouped very closely with several other PVY^{Eu-N} sequences from this state, whereas isolates WWA150131_G4_8 and Mex43 were more distant from both the Montana and UK PVY^{Eu-N} sequences. Thus, although the nomenclature Y^{Eu-N} and Y^{NA-N} is used here, it no longer reflects the geographical origins of isolates within each of these minor phylogroups. Three tomato and one potato PVY isolate sequences from Colombia (LaUnionT, VarA, VarB, and mar7) all fitted within the new South American minor phylogroup N2 recently reported by Fuentes et al. (2019). To assess the robustness of this newly defined South American minor phylogroup, we determined mean group distances within (i) the minor phylogroups defined in Green et al. (2018) and N2 (Table 3), and (ii) between other minor PVY phylogroups (Supplementary Table S1). The genetic distances within each of minor phylogroups PVY^{Eu-N}, PVY^{NA-N}, and PVY^{N2} were 3 to 8 times lower than between each of them. Similarly, within minor phylogroups PVY^{O-O} and PVY^{O-O5}, the same mean within phylogroup distances were 2 to 3 times lower than those between PVY^{O-O} and PVY^{O-O5}. These findings support separation of PVY^{N2} and PVY^{O-O5} within major phylogroups PVY^N and PVY^O, respectively (Fig. 1).

Our study confirms that the classical and much studied UK isolate Y^{C-R} from 1943 (Bawden and Kassanis 1947; Jones 1990; Kassanis and Govier 1971; Kehoe and Jones 2011, 2016) fits closely with classical Netherlands isolate Y^{C-PRI-509} (EU563512) from 1938 (Dulleman et al. 2011) within minor phylogroup PVY^{C1}. As mentioned above, PVY^{C1} contains isolates from many solanaceous species (Dulleman et al. 2011; Fuentes et al. 2019; Gibbs et al. 2017; Green et al. 2017b; Kerlan and Moury 2008; Quenouille et al. 2013). This finding is significant as, apart from Y^{C-PRI-509}, all other PVY^C sequences from potato fit within minor phylogroup PVY^{C2} (Green et al. 2017b; Jones and Kehoe 2016). Our research also identifies the oldest examples of complete genomic sequences belonging to minor phylogroups PVY^{NA-N} and PVY^{Eu-N}, as a newly sequenced historical UK Y^N isolate belonged to each of them. This shows that both PVY^{NA-N} and PVY^{Eu-N} were present in UK in the early 1980s. Our four new examples of modern nonrecombinant PVY isolates from Mexico and the U.S.A. were all in minor phylogroup PVY^{Eu-N} (Fig. 1). Moreover, our study and that of Kehoe and Jones (2016) agree in finding no recombinant PVY isolates in the UK in the first half of the 1980s as neither the eight genomes sequenced previously from UK isolates from 1943 to 1984 nor the four PVY isolates from that era sequenced in our study were recombinants. This suggests that much of the sequence diversity now known to occur among nonrecombinant PVY isolates was already present in the UK by 1984. Similarly, neither the five recently sequenced PVY isolates from Western Australia obtained by Kehoe and Jones (2016) nor the one sequenced here were recombinants, suggesting recombinant PVY has not yet arrived in this isolated world region. Furthermore, our study found all three historical PVY^Z strain group

Table 3. Mean group distances within minor phylogroups of potato virus Y

Minor phylogroup	Within strain - mean group distance	
	d ^a	Standard error
C	0.100	0.003
Chile3	n/a	n/a
Eu-N	0.008	0.001
NA-N	0.007	0.000
O	0.018	0.001
O5	0.014	0.001
Outgroup	n/a	n/a
N2	0.017	0.001

^a Distances between minor phylogroups defined by Green et al. (2018), and Colombian sequences within South American minor phylogroup N2 defined by Fuentes et al. (2019). Distances were produced in MEGA 7 using the maximum likelihood method with 100 bootstrap replicates. Model selected as best by MEGA 7: GTR+I+G (12 Gamma categories). n/a = not applicable as only one isolate present.

nonrecombinant isolates (Y^Z-CM2, Y^Z-CRM2, Y^Z-DS) to be within minor phylogroup PVY^{O-O}, and three of the four modern Australian isolates to be within minor phylogroup PVY^{O-O5}; Kehoe and Jones (2016) had previous misplaced five historical PVY^{O-O} isolates (Y^Z-CM2, Y^Z-DS, Y^O-CRN, Y^O-KE, Y^O-RS) within PVY^{O-O5}.

In 1984, Beczner et al. (1984) first reported presence of PTNRD caused by PVY in Hungary. When European PVY isolates associated with PTNRD were sequenced, they proved to be recombinants between PVY^O and PVY^N (Boonham et al. 1999, 2002; Glais et al. 1998, 2002; Revers et al. 1996). After the 1980s, recombinant PVYs largely displaced nonrecombinants in Europe, and after that in North America and many other world regions (Funke et al. 2017; Karasev and Gray 2013a, b). The lack of detection of any recombinants among the 11 historical PVY^O and PVY^N isolates first obtained in 1981 to 1984 (Table 1) suggest they were absent or uncommon in the UK at that time. The same is also likely for the rest of Europe.

Why do the two oldest potato PVY sequences occur in otherwise non-potato minor phylogroup PVY^{C1}? These two isolates were obtained in the Netherlands or UK in 1939 and 1943, respectively, i.e., 77 to 81 years ago (Table 1). Apparently, such potato isolates were relatively common then, but disappeared subsequently as none were found in the 1980s when several PVY^{C2} isolates were detected in the UK (Jones 1990; Kehoe and Jones 2011, 2016) and none were found subsequently. Greater abundance of hypersensitivity gene *Nc* in potato cultivars after the 1940s might explain this apparent disappearance, but this explanation is inconsistent with the ease of finding isolates in minor phylogroup PVY^{C2} in the UK in the early 1980s (Cockerham 1943, 1970; Jones 1990). Another possible explanation is that, being less well adapted to infect potato, PVY^{C1} isolates were displaced by PVY^{C2} isolates in potato cultivars without gene *Nc*. The predominance of isolates infecting tobacco, pepper, tomato, and other solanaceous crops in PVY^{C1} (Fuentes et al. 2019; Gibbs et al. 2017; Green et al. 2017b; Kehoe and Jones 2016; Kerlan and Moury 2008; Moury 2010; Quenouille et al. 2013) seems consistent with this explanation.

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