Comparative genomics of Pseudomonas syringae reveals convergent gene gain and loss associated with specialization onto cherry (Prunus avium)
Hulin, Michelle T.; Armitage, Andrew D.; Vicente, Joana G.; Holub, Eric B.; Baxter, Laura; Bates, Helen J.; Mansfield, John W.; Jackson, Robert W.; Harrison, Richard J.

DOI: 10.1111/nph.15182

License: Creative Commons: Attribution (CC BY)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Hulin, MT, Armitage, AD, Vicente, JG, Holub, EB, Baxter, L, Bates, HJ, Mansfield, JW, Jackson, RW & Harrison, RJ 2018, ‘Comparative genomics of Pseudomonas syringae reveals convergent gene gain and loss associated with specialization onto cherry (Prunus avium)’, New Phytologist, vol. 219, no. 2, pp. 672-696. https://doi.org/10.1111/nph.15182

Link to publication on Research at Birmingham portal

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• User may use extracts from the document in line with the concept of ‘fair dealing’ under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 03. Nov. 2020
Comparative genomics of *Pseudomonas syringae* reveals convergent gene gain and loss associated with specialization onto cherry (*Prunus avium*).

Michelle T. Hulin1,2, Andrew D. Armitage1, Joana G. Vicente3, Eric B. Holub3, Laura Baxter3, Helen J. Bates1, John W. Mansfield4, Robert W. Jackson2 and Richard J. Harrison1,2

1NIAB EMR, New Road, East Malling, ME19 6BJ, UK; 2School of Biological Sciences, University of Reading, Reading, RG6 6AJ, UK; 3School of Life Sciences, Warwick Crop Centre, University of Warwick, Wellesbourne, CV35 9EF, UK; 4Faculty of Natural Sciences, Imperial College London, London, SW7 2AZ, UK

Author for correspondence: Richard J. Harrison
Tel: +44 (0)1732 843833
Email: richard.harrison@emr.ac.uk

Received: 9 January 2018
Accepted: 22 March 2018

Key words: avirulence, bacterial canker, comparative genomics, host specialization, prediction, *Pseudomonas*, toxins, type III effectors.

Introduction

*Pseudomonas syringae* is a species complex, associated with plants and the water cycle, comprising several divergent clades that frequently recombine (Young, 2010; Parkinson *et al.*, 2011; Berge *et al.*, 2014; Baltrus *et al.*, 2017). It is a globally important pathogen, causing disease on over 180 different host species. *P. syringae* is responsible for recurring chronic diseases in perennial crops, such as cherry canker (Lamichhane *et al.*, 2014), and also sporadic outbreaks on annual crops, such as bacterial speck of tomato (*Solanum lycopersicum*) (Şahin, 2001). Individual strains are reported to be specialized and assigned to over 60 host-specific pathovars; some of these are further divided into races which show host genotype specificity (Joardar *et al.*, 2005). Strains also exist that can infect a variety of crop species, indicating that specialization is not always the norm (Monteil *et al.*, 2013; Bartoli *et al.*, 2015a,b). This complexity makes *P. syringae* an important model to study the evolution of host specificity (O’Brien *et al.*, 2011; Mansfield *et al.*, 2012).

High-throughput sequencing has become a major tool in bacteriology (Edwards & Holt, 2013). With the increasing number of genomes available, population-level analyses allow complex evolutionary questions to be addressed, such as how disease epidemics emerge and what ecological processes drive the evolution of pathogenicity (Guttman *et al.*, 2014; Monteil *et al.*, 2016). Before genomic methods were available, mutational studies of *P. syringae* were used to identify functional virulence factors in pathogenesis, such as type III secretion system effector (T3E) repertoires and toxins (Lindgren, 1997; Bender *et al.*, 1999). Some T3Es were also shown to act as avirulence (*avr*) factors when detected by a corresponding pathogen recognition (R) protein in the host, leading to effector-triggered immunity (ETI) (Jones & Dangl, 2006). ETI is often associated with the hypersensitive response (HR) which is a cell death mechanism important in preventing pathogen spread (Morel & Dangl, 1997). Evidence suggests that *P. syringae* has evolved a functionally redundant repertoire of effectors, which is postulated to allow pathogen populations to lose/modify expendable *avr* elicitors, with minimal impact on overall virulence (Arnold & Jackson, 2011). It is proposed that as pathogen lineages specialize, they fine-tune their effector repertoires to maximize fitness in this niche by ensuring adequate growth and transmission, whilst avoiding detection by the plant immune system. Host range becomes restricted because the pathogen may lose effectors important for disease on other hosts or gain effectors detected by other plant species (Schulze-
Lefert & Panstruga, 2011). Many genomics studies have therefore focused on linking variation in virulence gene complements with particular diseases (Baltrus et al., 2011, 2012; O’Brien et al., 2012).

Much of the understanding of P. syringae–plant interactions has been achieved using herbaceous plant models. Woody pathosystems provide a greater challenge (Lamichhane et al., 2014). Population genomics of P. syringae pv actinidiae, the causal agent of kiwifruit canker, revealed that three pathogenic clades, with distinct effector sets, have arisen during kiwifruit cultivation (McCann et al., 2013, 2017). A study of the olive pathogen P. syringae pv savastanoi revealed that the hopBL effector family is overrepresented in wood-infecting pathovars (Matas et al., 2014). Genes involved in the metabolism of aromatic compounds, phytohormone production, tolerance to reactive oxygen species and sucrose metabolism have also been associated with virulence on woody tissues (Green et al., 2010; Bartoli et al., 2015b; Buonaurio et al., 2015; Nowell et al., 2016).

This study used genomics to examine the evolution of strains that cause bacterial canker on sweet and wild cherry (both Prunus avium). Clades of P. syringae that constitute the main causal agents of bacterial canker include P. syringae pv morsprunorum (Psm) race 1 and race 2 and P. syringae pv syringae (Ps) (Bull et al., 2010; Bultreys & Kaluzna, 2010). In addition, P. syringae pv avii causes bacterial canker of wild cherry (Ménard et al., 2003). The cherry-pathogenic clades of P. syringae are reported to exhibit differences in virulence, host range and lifestyle (Crosse & Garrett, 1966; Scottichini, 2010), making the P. syringae–cherry interaction a good pathosystem to study convergent gain of pathogenicity. The genomic analysis has been coupled with robust pathogenicity testing (Hulin et al., 2018) and functional analysis of potential avirulence (avr) genes. This study provides a proof of concept that genomics-based methods can be used to identify candidate genes involved in disease and will likely become the major tool in disease monitoring, diagnostics and host range prediction.

Materials and Methods

Bacteria, plants and pathogenicity tests

Methods used for bacterial culture and sources of plants were as described in Hulin et al. (2018) and are detailed in Supporting Information Methods S1. Plant species utilized included P. avium L. and Nicotiana tabacum L. Pseudomonas strains are listed in Table 1. Escherichia coli was grown on lysogeny broth (LB) agar plates and grown overnight at 37°C. Antibiotic concentrations: kanamycin, 50 μg ml⁻¹; gentamycin, 10 μg ml⁻¹; spectinomycin, 100 μg ml⁻¹; nitrofurantoin, 100 μg ml⁻¹. X-gal was used at a concentration of 80 μg ml⁻¹. Table S1–S3 list the P. syringae mutants, plasmids and primers used in this study.

Pathogenicity tests, performed on detached cherry leaves and woody tissues, were aligned using CLUSTALW (Larkin et al., 2007) and trimmed using GROK (Castrasana, 2000). Gene alignments were concatenated using GENEIOUS (Kececi et al., 2012). RAxML-AXX v.8.1.15 (Stamatakis, 2014) was used in partitioned mode to build the maximum likelihood phylogeny, with a general time reversible (GTR) gamma model of substitution and 100 non-parametric bootstrap replicates. To detect core genes that may have undergone recombination, the program GENECONV (Sawyer, 1989) was used as in Yu et al. (2016). Whole-genome alignments were performed using PROGRESSIVE_MAUVE (Darling et al., 2010).

Genome sequencing, assembly and annotation

Bioinformatics commands for analyses performed in this paper are available on Github (https://github.com/harrisonlab/pseudomonas). Genome sequencing using Illumina and genome assembly were performed as in Hulin et al. (2018). For long-read sequencing, PacBio (Pacific Biosystems, Menlo Park, CA, USA) and MinION (Oxford Nanopore, Oxford, UK) were used. High molecular weight DNA was extracted using a cetyltrimethylammonium bromide method (Feil et al., 2012). For the PacBio sequencing of strains R1-5244, R2-leaf and syr9097, DNA samples were sent to the Earlham Institute (Norwich) for PacBio RSII sequencing.

For MinION sequencing of Psm R1-5300, the DNA library was prepared using the RAD001 rapid-prep kit (Oxford Nanopore) and run on the Oxford Nanopore MinION, flowcell vR9.5 followed by basecalling using METRICHOR (Oxford Nanopore). MinION reads were extracted from FAST5 files using PORETOOLS (Loman & Quinlan, 2014). The sequencing reads for both long-read technologies were trimmed and assembled using CANU (Berlin et al., 2015), and CIRCULATOR was used to circularize contigs (Hunt et al., 2015). The assemblies were polished using error-corrected Illumina reads with BOWTIE2, SAMTOOLS and PILON 1.17 (Li et al., 2009; Langmead & Salzberg, 2013; Walker et al., 2014).

Plasmid profiling was performed using an alkaline-lysis method and gel electrophoresis (Moulton et al., 1993; Neale et al., 2013). Genomes were submitted to GenBank and accession numbers are listed in Table 1.

Orthology analysis

OrthoMCL (Li et al., 2003) was used to identify orthologous genes. All genomes were re-annotated using RAST (Aziz et al., 2008) to ensure similar annotation quality. For this reason, the Illumina short-read assemblies of the four long-read sequenced genomes (R1-5244, R1-5300, R2-leaf and syr9097) were used in orthology analysis. OrthoMCL was run with default settings and a 50 residue cut-off length. All RAST annotated protein files used in this analysis are available on Github (https://github.com/harrisonlab/pseudomonas/).

Phylogenetic and genomic analysis of Pseudomonas syringae

Nucleotide and genomic sequences of Pseudomonas syringae present in all genomes were aligned using CLUSTALW (Larkin et al., 2007) and trimmed using GROK (Castrasana, 2000). Gene alignments were concatenated using GENEIOUS (Kececi et al., 2012). RAxML-AXX v.8.1.15 (Stamatakis, 2014) was used in partitioned mode to build the maximum likelihood phylogeny, with a general time reversible (GTR) gamma model of substitution and 100 non-parametric bootstrap replicates. To detect core genes that may have undergone recombination, the program GENECONV (Sawyer, 1989) was used as in Yu et al. (2016). Whole-genome alignments were performed using PROGRESSIVE_MAUVE (Darling et al., 2010).

Virulence and mobility gene identification

All T3E-encoding protein sequences were downloaded from pseudomonas-syringae.org, including the recent classification of
**Table 1** List of bacterial strains used in this study, including a range of cherry pathogens and nonpathogens

| Strain   | Pathovar | Race | PG | Isolation source | Isolator | Prunus cv | Sequenced | Pathogenicity tested on cherry (Prunus avium) | Accession number |
|----------|----------|------|----|------------------|----------|-----------|-----------|-----------------------------------------------|------------------|
| avii5271 | avii     | 1    | 3  | garlic           | Garrett, 1990, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBAO00000000000 |
| R1-5270  | morsprunorum | 1    | 3  | garlic           | Garrett, 1990, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBAN00000000000 |
| R2-7968A | morsprunorum | 2    | 1  | garlic           | Vicente, 2000, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBAI00000000000 |
| R2-9095  | morsprunorum | 2    | 1  | garlic           | Roberts, 2010, UK | Wild cherry | This work | M. Hulin, pers. obs.                          | MLED00000000000 |
| syr5264  | syringae  | 2    | 3  | garlic           | Garrett, 1990, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBAQ00000000000 |
| syr5275  | syringae  | 2    | 3  | garlic           | Garrett, 1990, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBP00000000000 |
| syr7928A | syringae  | 2    | 3  | garlic           | Vicente, 2000, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBAL00000000000 |
| syr8094A | syringae  | 2    | 3  | garlic           | Vicente, 2001, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBAK00000000000 |
| Ps-7928C | Unknown   | 2    | 3  | garlic           | Vicente, 2000, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBM00000000000 |
| Ps-7969  | Unknown   | 2    | 3  | garlic           | Vicente, 2000, UK | Wild cherry | This work | Vicente et al. (2004)                          | NBJ00000000000 |
| R1-5244  | morsprunorum | 1    | 3  | garlic           | Crosse, 1960, UK | Unknown    | This work | Hulin et al. (2018)                           | CP026557–CP026561 |
| R1-5300  | morsprunorum | 1    | 3  | garlic           | Prunier, UK | Victoria  | This work | Hulin et al. (2018)                           | MLEN00000000000 |
| R2-leaf  | morsprunorum | 2    | 1  | garlic           | Napoelen     | Victoria  | This work | Hulin et al. (2018)                           | CP026562–CP026567 |
| syr9097  | syringae  | 2    | 3  | garlic           | Roberts, 2010, UK | Unknown    | This work | Hulin et al. (2018)                           | CP026568 |
| syr2675  | syringae  | 2    | 3  | Phaseolus vulgaris | 1965, Kenya | Unknown    | This work | Hulin et al. (2018)                           | MLEX00000000000 |
| syr2676  | syringae  | 2    | 3  | Phaseolus vulgaris | 1990, Lesotho | Unknown    | This work | Hulin et al. (2018)                           | MLEY00000000000 |
| syr2682  | syringae  | 2    | 3  | Phaseolus vulgaris | 1990, Lesotho | Unknown    | This work | Hulin et al. (2018)                           | MLFA00000000000 |
| syr3023  | syringae  | 2    | 3  | Syringa vulgaris  | 1950, UK | Unknown    | This work | Hulin et al. (2018)                           | MLFD00000000000 |
| syr100   | syringae  | 2    | 3  | Phaseolus lunatus | 1962, Kenya | Unknown    | This work | Hulin et al. (2018)                           | MLEV00000000000 |
| R1-9326  | morsprunorum | 1    | 3  | Prunus domestica | Roberts, 2011, UK | Victoria  | This work | Hulin et al. (2018)                           | MLEO00000000000 |
| R1-9629  | morsprunorum | 1    | 3  | Prunus domestica | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLP00000000000 |
| R1-9646  | morsprunorum | 1    | 3  | Prunus domestica | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLEE00000000000 |
| R1-9657  | morsprunorum | 1    | 3  | Prunus domestica | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLF00000000000 |
| R2-5255  | morsprunorum | 2    | 3  | Prunus domestica | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLEC00000000000 |
| R2-5260  | morsprunorum | 2    | 3  | Prunus domestica | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLEG00000000000 |
| R2-SC214 | morsprunorum | 2    | 3  | Prunus domestica | Roberts, 1983, UK | Wild cherry | This work | Hulin et al. (2018)                           | MLEI00000000000 |
| syr9293  | syringae  | 2    | 3  | Prunus domestica | Roberts, 2011, UK | Victoria  | This work | Hulin et al. (2018)                           | MLEQ00000000000 |
| syr9630  | syringae  | 2    | 3  | Prunus domestica | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLER00000000000 |
| syr9644  | syringae  | 2    | 3  | Prunus avium     | Roberts, 2012, UK | Stella    | This work | Hulin et al. (2018)                           | MLEK00000000000 |
| syr9654  | syringae  | 2    | 3  | Prunus avium     | Roberts, 2012, UK | Victoria  | This work | Hulin et al. (2018)                           | MLE50000000000 |
| syr9656  | syringae  | 2    | 3  | Prunus avium     | Roberts, 2012, UK | Kiku-Shidare | This work | Hulin et al. (2018)                           | MLEM00000000000 |
| syr9659  | syringae  | 2    | 3  | Prunus avium     | Roberts, 2012, UK | Kiku-Shidare | This work | Hulin et al. (2018)                           | MLEL00000000000 |
| Strain        | Pathovar | Race | PG | Isolation source | Prunus cv | Isolator                  | Pathogenicity tested on cherry (Prunus avium) | Accession number |
|--------------|----------|------|----|------------------|-----------|---------------------------|-----------------------------------------------|------------------|
| Ps-9643      | Unknown  | 1    |     | Prunus domestica |           | Roberts, 2012, UK         | Wild cherry                                   | MLET00000000     |
| avii3846     | avii     | 1    | 1  | Prunus avium     |           | 1991, France              | Hulin et al. (2018)                           | LIIJ00000000     |
| R1-2341      | morsprunorum | 1  | 3  | Prunus cerasus   |           | 1988, Hungary             | Nowell et al. (2016)                          | LIIB00000000     |
| R1-5269      | morsprunorum | 1  | 3  | Prunus cerasus   |           | Garrett, 1990, UK         | Wild cherry                                   | LIHZ00000000     |
| R2-5261      | morsprunorum | 2  | 1  | Prunus avium     |           | Garrett, UK               | Nowell et al. (2016)                          | LIA00000000      |
| R2-302280    | morsprunorum | 1  |     | Prunus domestica |           | USA                       | Baltrus et al. (2011)                         | AEAE00000000     |
| syr2339      | syringae | 2    | 2  | Prunus avium     |           | 1984, Hungary             | Nowell et al. (2016)                          | LIHU00000000     |
| syr7872      | syringae | 2    | 2  | Prunus domestica |           | Lewis, 2000, UK           | Wild cherry                                   | LIHS00000000     |
| syr7924      | syringae | 2    | 2  | Prunus avium     |           | Vicente, 2000, UK         | Nowell et al. (2016)                          | LIHR00000000     |
| acer302273   | aceris   | 2    |     | Acer sp.         |           | USA                       | Baltrus et al. (2011)                         | AEOA00000000     |
| acti18884    | actinidiae | 1   | 1  | Actinidia deliciosa | 2010, New Zealand   | McCann et al. (2013)        | nt                                             | AOKO00000000     |
| acti19073    | actinidiae | 1   | 1  | Actinidia deliciosa | 1998, Korea     | McCann et al. (2013)        | nt                                             | AOJR00000000     |
| acti212056   | actinidiae | 1   | 1  | Actinidia deliciosa | 2012, Japan     | Sawada et al. (2015)        | nt                                             | BBWG00000000     |
| acti302091   | actinidiae | 1   | 1  | Actinidia deliciosa | 1984, Japan     | Baltrus et al. (2011)       | nt                                             | AEAL00000000     |
| actICRAFRU   | actinidiae | 1   | 1  | Actinidia deliciosa | 2010, Italy    | Butler et al. (2013)        | nt                                             | ANGD00000000     |
| actiNCPPB3871| actinidiae | 1   | 1  | Actinidia deliciosa | 1992, Italy    | Marcelletti et al. (2011)   | nt                                             | ANGD00000000     |
| aes089323    | aesculi  | 3    | 3  | Aesculus hippocastanum | India, 1980 | Baltrus et al. (2011)       | nt                                             | AEAD00000000     |
| aes2250      | aesculi  | 3    | 3  | Aesculus hippocastanum | 2008, UK      | Green et al. (2010)         | nt                                             | ACXT00000000     |
| aes3681      | aesculi  | 3    | 3  | Aesculus hippocastanum | 1969, India   | Green et al. (2010)         | nt                                             | ACSX00000000     |
| amy3205      | amygdali | 3    | 3  | Prunus dulcis     | 1967, Greece  | Bartoli et al. (2015a)      | nt                                             | JYHB00000000     |
| amyICMP3918  | amygdali | 3    | 3  | Prunus dulcis     | Panagopoulos, 1967, Greece | Thakur et al. (2016) | nt                                             | LJPQ00000000     |
| avelBP631    | avellanae | 1   | 2  | Corylus avellana | 1976, Greece | O’Brien et al. (2012)       | Hulin et al. (2018)                           | AKB50000000      |
| avelVe037    | avellanae | 2   | 2  | Corylus avellana | 1990, Italy  | O’Brien et al. (2012)       | nt                                             | ACKJ00000000     |
| BRIP34876    | Unknown  | 2    | 2  | Hordeum vulgare  | 1971, Australia | Gardiner et al. (2013)      | nt                                             | AMXX00000000     |
| castCFBP4217 | castaneae | 3   | 3  | Castanea crenata | 1977, Japan  | Nowell et al. (2016)        | nt                                             | LIHH00000000     |
| CC1416       | Unknown  | 1    |     | Epilithon        | USA         | Baltrus et al. (2014b)      | nt                                             | AVEP00000000     |
| CC1544       | Unknown  | 1    |     | Lake water       | France      | Baltrus et al. (2014b)      | nt                                             | AVEI00000000     |
| CC1559       | Unknown  | 1    |     | Snow             | France      | Baltrus et al. (2014b)      | nt                                             | AVEG00000000     |
| CC94         | Unknown  | 2    |     | Cantaloupe       | France      | Baltrus et al. (2014b)      | nt                                             | AVEA00000000     |
| Strain       | Pathovar  | Race | PG | Isolation source         | Isolator | Prunus cv          | Sequenced              | Pathogenicity tested on cherry (Prunus avium) | Accession number   |
|-------------|-----------|------|----|--------------------------|----------|--------------------|------------------------|----------------------------------------------|--------------------|
| cera6109    | cerascola | 3    | Prunus yedoensis          | 1995, Japan | Nowell et al. (2016) | nt                      | LIIG000000000          |
| ceralCMP17524 | cerascola | 3    | Prunus yedoensis          | Japan    | Thakur et al. (2016) | nt                      | LJQA000000000          |
| ciclCMP5710  | cicaronei | 3    | Ceratonia siliqua         | Italy    | Thakur et al. (2016) | nt                      | LJPY000000000          |
| cunnICMP11894 | cunninghamiae | 3 | Cunninghamia lanceolata | China | Thakur et al. (2016) | nt                      | LJQE000000000          |
| daphlCMP9757 | daphniphylli | 3 | Daphniphyllum tejsmannii | Japan    | Thakur et al. (2016) | nt                      | LQJF000000000          |
| delphi569    | delphinii | 1    | Delphinium sp.            | New Zealand | Thakur et al. (2016) | nt                      | LJQH000000000          |
| dendro3226   | dendropanacis | 3 | Dendropanax trifidus | 1979, Japan | Bartoli et al. (2015a) | nt                      | YJHG000000000          |
| dendro4219   | dendropanacis | 3 | Dendropanax trifidus | 1981, Japan | Bartoli et al. (2015a) | nt                      | YJHD000000000          |
| dendro9150   | dendropanacis | 3 | Dendropanax trifidus | Japan    | Thakur et al. (2016) | nt                      | LQJG000000000          |
| erio4455     | eriobotryae | 3  | Eriobotrya japonica | USA      | Thakur et al. (2016) | nt                      | LQII000000000          |
| glyR4        | glycinea  | 3    | Glyce max                | Cross, 1960, USA | Qi et al. (2011) | nt                      | AEQH000000000          |
| ICMP19498    | Unknown   | 3    | Actinidia deliciosa      | 2010, New Zealand | Visnovsky et al. (2016) | nt                      | LKCH000000000          |
| lach301315   | lachrymans | 3   | Cucumis sativus          | Japan    | Baltrus et al. (2011) | nt                      | AEA000000000          |
| lach302278   | lachrymans | 1   | Cucumis sativus          | USA      | Baltrus et al. (2011) | nt                      | AEA000000000          |
| lapsaICMP3947 | lapsa    | 2    | Zea sp.                  | Unknown  | Thakur et al. (2016) | nt                      | LJQK000000000          |
| meli6289     | meliae    | 3    | Melia azedarach          | Japan    | Thakur et al. (2016) | nt                      | LJQT000000000          |
| morsU7805    | morsprunorum | 3  | Prunus mume              | Unknown  | Mott et al. (2016) | nt                      | LGLO000000000          |
| myriAZ8448   | myricae   | 3    | Myrica rubra             | Japan    | Thakur et al. (2016) | nt                      | LGLA000000000          |
| neriiICMP16943 | savastanoi | 3  | Olea europea            | Spain    | Thakur et al. (2016) | nt                      | LJQW000000000          |
| paniLMG2367  | panici    | 2    | Panicum miliaceum        | Unknown  | Liu et al. (2012) | nt                      | ALAC000000000          |
| papu1754     | papulans  | 2    | Malus sylvestris         | 1973, Canada | Nowell et al. (2016) | nt                      | JYHI000000000          |
| persNCPPB2254 | persicae | 1    | Prunus persica          | 1972, France | Zhao et al. (2015) | nt                      | LAZV000000000          |
| photICMP7840 | photiniae | 3    | Photinia glabra          | Japan    | Thakur et al. (2016) | nt                      | LQJO000000000          |
| pisiPP1      | pisi      | 2    | Pism sativum             | Japan    | Baltrus et al. (2014a) | nt                      | AUZRO000000000          |
| phas1448a    | phaseolicola | 3  | Phaseolus vulgaris       | Teversion, 1965, Ethiopia | Joardar et al. (2005) | nt                      | CP00000058            |
| rhapsFBP4220 | rhaphiolepis | 3   | Rhaphiolepis umbellata   | 1980, Japan | Nowell et al. (2016) | nt                      | LHV000000000          |
| RMA1         | Unknown   | 1    | Aquilegia vulgaris       | Jackson, 2012, UK | Hulin et al. (2018) | nt                      | MLEU000000000          |
| sava3335     | savastanoi | 3   | Olea europea            | Stead, France | Rodríguez-Palenzuela et al. (2010) | nt                      | ADMIO000000000          |
| sava4352     | savastanoi | 3   | Olea europea            | Yugoslavia | Thakur et al. (2016) | nt                      | LGKRO000000000          |
| Strain          | Pathovar | Race | PG | Isolation source | Prunus cv | Sequenced                          | Pathogenicity tested on cherry (Prunus avium) | Accession number |
|----------------|----------|------|----|------------------|-----------|------------------------------------|-----------------------------------------------|------------------|
| savaDAPP-PG722  | savastanol | 3    | Olea europaea | Italy       | 1985, Hungary | Moretti et al. (2014) | nt | JOJV000000000 |
| savaPseNe107    | savastanol | 3    | Olea europaea | Balestra, Nepal | 2011, France | Bartoli et al. (2015a) | nt | JYHF000000000 |
| soliCMP16925    | solidagae | 2    | Solidago altissima | Japan | 1960, UK | Thakur et al. (2016) | nt | JYHF000000000 |
| syr1212         | syringae  | 2    | Pisum sativum | UK | 1986, Italy | Baltrus et al. (2014a) | nt | AVCR000000000 |
| syr2340         | syringae  | 2    | Prunus sp. | 1985, Hungary | 2011, France | Nowell et al. (2016) | nt | LIHT000000000 |
| syr41a          | syringae  | 2    | Prunus armeniaca | 1986, UK | 1985, France | Bartoli et al. (2015a) | nt | JYHJ000000000 |
| syrB301D        | syringae  | 2    | Prunus communis | 1960, UK | 1960, UK | Ravindran et al. (2015) | nt | CP005969 |
| syrB64          | syringae  | 2    | Triticum aestivum | 1985, USA | 1985, France | Duknik & Dudler (2014) | nt | ANZF000000000 |
| syrB728a        | syringae  | 2    | Phaseolus vulgaris | 1987, USA | 1980, Australia | Feil et al. (2005) | nt | CP000075 |
| syrHS191        | syringae  | 2    | Panicum miliaceum | 1986, Canada | 1969, UK | Ravindran et al. (2015) | nt | CP006256 |
| syrUMAF0158     | syringae  | 2    | Mangifera indica | Cazorla, 2010, Spain | 1986, Canada | Martinez-Garcia et al. (2015) | nt | CP005970 |
| thea3923        | theae     | 1    | Camelia sinensis | 1974, Japan | 1974, Japan | Mazzaglia et al. (2012) | nt | AGNN000000000 |
| tomaDC3000      | tomato    | 1    | Solanum lycopersicum | 1960, UK | 1960, UK | Buell et al. (2003) | nt | AE016853 |
| tomaT1          | tomato    | 1    | Solanum lycopersicum | 1986, Canada | 1986, Canada | Almeida et al. (2009) | nt | ABSM000000000 |
| U8303           | Unknown   | 2    | Lake water | France | France | Baltrus et al. (2014b) | nt | AVD200000000 |
| ulmICMP3962     | ulmi      | 3    | Ulmus sp. | Yugoslavia | Yugoslavia | Thakur et al. (2016) | nt | LJRQ000000000 |
| USA007          | Unknown   | 1    | Stream water | USA | USA | Baltrus et al. (2014b) | nt | AVDY000000000 |
| USA011          | Unknown   | 1    | Stream water | USA | USA | Baltrus et al. (2014b) | nt | AVDX000000000 |

Pathovar designation, phylogroup, isolation information, cherry pathogenicity (reference for when tested; nt, not tested), publication of genome sequence and NCBI accession numbers are listed. Strains in bold were considered pathogenic in cherry. cv, cultivar of sweet cherry or plum. Long-read sequenced genomes are highlighted with shading. Strains are ordered, first with those sequenced in this study, followed by other *Pseudomonas* strains from cherry and plum used in plasmid profiling analysis and previously pathogenicity tested in Hulin et al. (2018). Next, further strains isolated from cherry and plum sequenced elsewhere are listed. Finally, the remaining strains were only used in comparative analysis. Note that all 108 genomes were used in initial orthology analysis but only 102 were used in the final phylogeny and comparative genomics.

*The pathogenic status of MAFF302280 on cherry is debated. This strain is reported to be the pathotype strain of *P. syringae pv morsprunorum* (*Psm*; Sawada et al., 1999), so is assumed to be equivalent to CFBP 2351, NCPPB2995, ICMP5795 and LMG5075. The strain NCPPB2995 was reported to be potentially nonpathogenic (Gardan et al., 1999). Whilst, the ‘same’ strain LMG5075 tested positive for pathogenicity in a recent publication (Gilbert et al., 2019). There is no definite link showing that MAFF302280 is the same strain as the others listed as it is not linked to them in online databases (http://www.straininfo.net/) or taxonomy-focused publications (Bull et al., 2010). It is assumed to be putatively pathogenic in this study owing to its close relatedness to other *Psm* R2 strains; however, further pathogenicity tests would be required to fully confirm this.*

HopF effectors into four alleles (Lo et al., 2016). tBLASTN (Altschul et al., 1990) was used to search each genome for homologues with a score of ≥ 70% identity and ≥ 40% query length to at least one sequence in each effector family. Nucleotide sequences were extracted and manually examined for frameshifts or truncations. Disrupted genes were classed as pseudogenes. A heatmap of effector presence was generated using heatmap.2 in gplots (Warnes et al., 2016). Interproscan (Quevillon et al., 2005) identified protein domains, and Illustrator for Biological Sequences was used for visualization (Liu et al., 2015). Genomic regions containing effectors were aligned using MAFFT (Katoh et al., 2002).
A similar analysis was performed for phytoxin and auxin biosynthesis, wood degradation, ice nucleation and plasmid-associated genes. Protein sequences were obtained from NCBI (Table S4) and blasted against the genome sequence as noted earlier. Prophage identification was performed using PHASTER (Arndt et al., 2016).

Gain and loss analysis

Gain loss mapping engine (GLOOME) was used to plot the gain and loss of genes on the core-genome phylogeny (Cohen et al., 2010). Effector genes were considered present even if predicted to be pseudogenes, as these can still be gained and lost. The optimization level was set to ‘very high’, a mixture model allowing variable gain and loss distributions was used and the distribution type was GENERAL_GAMMA_PLUS_INV. Highly probable events ($P \geq 0.80$) on the branches leading to cherry-pathogenic strains were extracted.

BayesTraits analysis

BayesTraits v.2 was used to correlate T3E gene evolution with pathogenicity (Pagel, 2004). A binary matrix was created of effector family presence and pathogenicity of each strain. The effector matrix was collapsed into effector families, as the different alleles likely perform similar biological functions in planta (Cunnac et al., 2011). Putative pseudogenes were considered absent, as they may be nonfunctional. The BayesTraits methodology followed an approach as in Press et al. (2013) and is described in detail in Methods S1.

Horizontal gene transfer analysis

For each effector family, best-hit nucleotide sequences were aligned using CLUSTALW (Larkin et al., 2007). RAxML was used to build a phylogenetic tree with a GTR model of evolution and 1000 bootstrap replicates. Incongruence with the core-genome tree was examined visually. To further assess horizontal transfer, a species–gene tree reconciliation method Ranger-DTL v.2 (Bansal et al., 2012) was used, as in Bruns et al. (2018). Full details are described in Methods S1.

Identification of genomic islands

Genomic islands (GIs) were identified in the PacBio-sequenced cherry pathogenic strains using IslandViewer3 (Dhillon et al., 2015). Islands were manually delimited as in McCann et al. (2013). BLASTN was utilized to determine if these GIs were present in other P. syringae genomes. As most GIs exceeded 10 kb and most genome assemblies were highly fragmented, the islands were split into 0.5 kb sections before analysis to prevent false negatives due to contig breaks. An island was concluded as likely to be present if all sections produced hits with a query length > 30%. To validate this approach, the Illumina-sequenced genome assemblies of the PacBio-sequenced strains were searched for their own islands.

General DNA manipulations and bacterial transformations

Cloning and other molecular biology techniques, including ectopic expression of potential avr genes, were as described in earlier studies (Staskawicz et al., 1984; Arnald et al., 2001; Kvitko & Collmer, 2011). Details are provided in Methods S1.

Results

Genome assembly and sequencing statistics

Eighteen P. syringae strains isolated from cherry and plum were phenotyped for pathogenicity and genome sequenced in a previous study (Hulin et al., 2018). To increase this sample, nine strains isolated from wild cherry and five additional non-Prunus out-groups were genome sequenced using the Illumina MiSeq. The genomes of eight cherry strains sequenced elsewhere (Baltrus et al., 2011; Nowell et al., 2016) were also downloaded from NCBI.

Information on the origin and pathogenicity of each strain is summarized in Table 1. Twenty-eight were considered pathogenic to cherry, including all Ps isolated from cherry and plum. By contrast, three Psm race 1 strains from plum (R1-5300, R1-9326 and R1-9629) and one from cherry, strain R1-9657, failed to induce canker on cherry following tree inoculations; and three strains of unknown taxonomy isolated from plum and cherry (Ps-9643, Ps-7928C and Ps-7960) were also non-pathogenic (references in Table 1). The cherry pathogens are referred to as their described pathovar names throughout this study. To simplify figures, cherry pathogens are highlighted and the first few letters of the pathovar name were used. ‘Ps’ becomes ‘syr’, as otherwise Ps could refer to other pathovars beginning with ‘s’ (e.g. savastanoi).

All strains included in this study were sequenced using Illumina MiSeq. Three representative strains (R1-5244, R2-leaf and syr9097) were sequenced using PacBio and the nonpathogenic Psm R1 strain R1-5300 was sequenced using Oxford Nanopore, to obtain more complete genomes. Table 2 summarizes the genome assembly statistics for all strains sequenced in this study and Hulin et al. (2018). Illumina genomes assembled into 23–352 contigs, whilst the long-read sequenced genomes assembled into one to six contigs. The number of plasmids in each strain was determined by plasmid profiling (Fig. S1). Psm R1 and R2 strains possessed between two and six plasmids, P. syringae pv avii 5271 possessed six plasmids, whereas, apart from three strains (syr5275, syr7928A, syr9644) with one plasmid each, most cherry-pathogenic strains of Ps did not possess plasmids. The strain syr9097, which was sequenced using PacBio, lacked plasmids. The genomes sequenced with long-read technology all assembled into the correct number of contigs corresponding to chromosome and plasmids, apart from R1-5300. The chromosome of this strain was separated into two contigs (tig0 and tig75), based on a whole-genome alignment with Psm R1 strain R1-5244 (Fig. S2).

The Psm R1 (R1-5244, R1-5300) and Psm R2 (R2-leaf) long-read assemblies revealed putatively complete plasmid contigs
containing plasmid-associated genes (Tables S5–S7). All three strains (R1-5244, R1-5300 and R2-leaf) possessed plasmids with repA homologues, indicating they may belong to common plasmid family pPT23A (Zhao et al., 2005). Several plasmids also contained T4SS conjugational machinery (VirB/D), so may be conjugative.

### Core-genome phylogenetic analysis

To examine the relatedness of strains, an analysis of core genes was carried out using 108 genomes of strains from the well-studied phylogroups 1–3 isolated from both plants and aquatic environments. A maximum likelihood phylogeny based on 1035 concatenated core genes was constructed (Fig. S3). There was low support for certain P2 and P3 clades based on bootstrap analysis. To determine if particular taxa were causing low support, the analysis was systematically repeated for the two phylogroups, with non-cherry strains removed. Support and tree likelihood values were compared (Table S8). Within P3, the removal of *P. syringae pv eriobotryae* or *P. syringae pv daphniphylli* improved support, whilst the removal of *P. syringae pv syringae* 1212 improved support values in P2 (Figs S4, S5). The global analysis was then repeated with these taxa removed (Figs S6–S9). The phylogeny, built using a 611 888 bp alignment, contained 102 taxa due to the removal of strains found to be identical to others (dendro4219, syr9630, R1-9629, R1-9326 and R1-5269). Most support values exceeded 70%, with good support for branches leading to cherry-pathogenic clades.

One explanation for the low support within P2 and P3 was that these clades have undergone core-genome recombination. The program GeneConv (Sawyer, 1989) showed that 140 genes had putatively recombined (127 288 bp total length, 20.8% of the alignment). Table S9 lists the number of recombination events per phylogroup. The most frequent core gene recombination occurred in P3 (73 genes affected), followed by 31 genes in P2 and only 13 in P1. Most support values exceeded 70%, with good support for branches leading to cherry-pathogenic clades.

Cherry pathogens are in bold. Long-read sequenced genomes are highlighted with shading. N50, the weighted median contig size in the assembly; Features, the number of protein encoding and RNA sequences in the RAST annotated genome.
Fig. 1 Core-genome phylogenetic tree. Multi-locus phylogeny based on 1035 genes which represent the core genome of *Pseudomonas syringae*. Strains from cherry and plum are highlighted in pink and blue respectively. Strains pathogenic to cherry (assessed in Hulin et al., 2018; Vicente et al., 2004) are labelled with red circles. Strains with long-read sequenced genomes are in black boxes. Phylogroups are also labelled for reference. Percentage bootstrap support values below 99% are shown for each node. The bar is nucleotide substitutions per site.

- Pathogenic on cherry
formed monophyletic clades. Within Psm R1, strains pathogenic to cherry formed a clade distinct from previously classified non-pathogenic strains (Hulin et al., 2018), indicating that there has been divergence in their core genomes. By contrast, Prunus-infecting strains of Ps were found across P2, interspersed with strains isolated from other plants and aquatic environments. To ensure that genomic comparisons between P2 strains were based on differential pathogenicity, several related non-Prunus strains were pathogenicity tested on cherry leaves. In planta bacterial populations of non-Prunus strains were reduced compared with Prunus Ps strains (Fig. S10; Table S10).

Search for virulence factors

The hrp pathogenicity island All sequenced strains contained the hrp pathogenicity island required for conventional Type III secretion. Core effector genes from the adjacent conserved effector locus (Alfano et al., 2000), such as avrE1, hopM1 and hopAA1, were present (Fig. S11). However, hopAA1 was truncated in both Psm R1 and R2 due to inversion events. The hopAA1 gene was truncated in Psm R2, whilst in Psm R1 both hopAA1 and hopM1 were truncated (Fig. S12).

Type III effectors and other virulence genes Genomes were then scanned for known virulence genes and a heatmap of presence, absence and pseudogenization was constructed (Fig. 2). In terms of T3Es, there was variation both between and within the different cherry-pathogenic clades. Notably, Psm R1, which contained strains pathogenic and nonpathogenic on cherry, showed clear differentiation in effector repertoires (Table S11). Psm R1, R2 and P. syringae pv avii possessed 24–34 effector genes, whereas Ps strains possessed nine to 15. The reduced effector repertoire of Ps was representative of P2 strains as previously noted (Baltrus et al., 2011; Dudnik & Dudler, 2014). Table 3 lists the effectors in each long-read genome assembly in order of appearance.

Non-T3 virulence factors were identified. All pathogenic Psm R1 strains possessed the coronatine biosynthesis clusters, which were plasmid borne in Psm R1-5244. All cherry-pathogenic Ps strains possessed at least one biosynthesis gene cluster for the toxins syringomycin, syringolin and syringopeptin, with several strains possessing all three. Strains within clade P2b possessed the biosynthesis genes for mangotoxin. The nonpathogenic cherry P2b strains Ps7928C and Ps7969 lacked all toxin biosynthesis clusters.

A cluster of genes named WHOP (woody hosts and Pseudomonas) thought to be involved in aromatic compound (lignin) degradation (Caballo-Ponce et al., 2016) was present in Psm R1 and R2, whilst P. syringae pv avii and most Ps strains contained no WHOP homologues. Two cherry P2d strains (syr2339 and syr7924) did, however, possess the catechol catBCA cluster. The genomes were also searched for the ice nucleation gene cluster. Members of Psm R1, Ps and P. syringae pv avii strains all possessed genes involved in ice nucleation (Fig. 2), whilst Psm R2 lacked the complete set of ice nucleation genes.

Associating type III effector evolution with host specificity

T3E evolution was statistically correlated with cherry pathogenicity, using BAYESTRAITS and GLOOME (Pagel, 2004; Cohen et al., 2010). BAYESTRAITS takes a binary matrix of two traits within a phylogeny and determines if changes in the two characters (effector gene and pathogenicity) have evolved independently or dependently. Fig. 3(a) shows the likelihood ratio of cherry pathogenicity being correlated with each effector family’s evolution, with significantly associated effectors highlighted.

BAYESTRAITS analysis using the core-genome phylogeny predicted the evolution of six T3E families was linked to cherry pathogenicity. These were hopBF, hopAB, hopH, hopAR, avrPto and hopBB. To account for any phylogenetic uncertainty, the program was also run 100 times on the full set of 100 bootstrapped trees generated by RAxML. The evolution of T3Es hopBF, hopAR and hopAB was always associated with pathogenicity for all 100 trees in all runs, indicating strong association. However, the T3E genes avrPto, hopBB and hopH were only significantly correlated for 88%, 77% and 62% of trees respectively, averaged across the different runs (Fig. S13). To determine how these genes had been gained or lost across the phylogeny, the program GLOOME was used (Cohen et al., 2010). Fig. 3(b) illustrates the predicted gain and loss of these T3Es on the branches leading to clades pathogenic to cherry. Those putatively associated with pathogenicity (high probability of gain in cherry-pathogenic clades) included hopAR1, hopBB1, hopBF1 and hopH1. The T3Es hopAB1 and avrPto1 were found to be lost from cherry pathogenic Psm R1, whilst the hopAB1 and hopAB3 alleles were pseudogenized in Psm R2 and P. syringae pv avii (Fig. 3b). All effector gain and loss events are presented in Fig. S14 and Table S12. Fig. S15 shows the phylogeny with branch labels used in GLOOME.

GLOOME predicted that key effectors have been gained in multiple clades. The hopAR1 gene has been gained in Psm R1, Psm R2, Ps and P. syringae pv avii. The T3E hopBB1 was present in the majority of strains within Psm R1, R2 and P. syringae pv avii but was absent from Ps strains. It showed high probability of gain on branches leading to both Psm R2 and P. syringae pv avii. However, GLOOME predicted loss in two Psm R1 strains, indicating that the gene may have experienced dynamic evolution in cherry pathogens. The hopBB1 effector is closely related to members of the hopF family and avrRpm2 (Lo et al., 2016). In addition to the significant acquisition of hopBB1 homologues, the hopF family was expanded in cherry pathogens. Pathogenic strains in Psm R1 and R2 all possessed two hopF alleles each (hopF3 and hopF4, and hopF2 and hopF4; see Fig. 2). P. syringae pv avii did not possess any hopF homologues, but had gained hopBB1. By contrast, Ps strains lacked all hopF members.

Origins of key effectors in cherry pathogens

To understand the origins of key effectors, gene phylogenies were produced. Incongruence with the core-genome phylogeny indicated that effector sequences had likely experienced horizontal
**Fig. 2** Virulence gene identification. (a) Heatmap of virulence gene presence and absence across *Pseudomonas syringae* (*avrA1-hopBJ1*). The dark green squares indicate presence of a full-length type III effector (T3E) homologue, whereas light green squares indicate that the gene is disrupted or truncated in some way. Strains isolated from cherry and plum are highlighted in pink and blue respectively. Asterisks indicate nonpathogenic to cherry in controlled pathogenicity tests. Strains with long-read sequenced genomes are in black boxes. The cherry-pathogenic clades are illustrated via horizontal shading of cells with *P. syringae pv morsprunorum* (*Psm*) R1 in blue, *Psm* R2 in light green, *P. syringae pv syringae* (*Pss*) in pink and *P. syringae pv avii* in orange. Strains are ordered based on the core-genome phylogenetic tree, which is represented by the dendrogram, with phylogroups labelled (P1–P3). (b) Continuation of (a) for T3Es *hopBK1–hopZ5* and additional non-T3 virulence factors, which are coloured in dark blue (all genes full-length and present) and light blue (not all genes present/truncation of genes). (c) The total number of full-length and pseudogenized T3E genes plotted for each strain, with cherry pathogenic strains in red and other strains in grey.
The sequences for the different cherry pathogenic clades did not cluster with each other, indicating convergent acquisition. Prophage identification (Table S14) did, however, reveal that this T3E is predicted to have been gained in Psm R1 and R2 within different phage sequences, whilst in Ps it is on a genomic island (Fig. 5b), and so has been acquired via distinct mechanisms. The Psm R1 phage is 51.5 kb, described as intact, and contains both hopARI and a truncated version of hopBK1. The Psm R2 phage sequence was 37.1 kb and was described as ‘incomplete’, indicating it did not have all the components of an active prophage. Further analysis of this region in Ps R1 and R2 strains revealed a shared adjacent tRNA-Thr gene (Fig. S19a,b). Within P2, although cherry Ps strains lacked the phage, several strains isolated from bean (syr2675, syr2676 and syr2682) possessed the hopARI gene within a phage homologous to that in Psm R2. The syr2675 hopARI sequence was also the most closely related homologue of Psm R2 hopARI (Fig. 5a). This evidence suggests that this effector gene may have been transferred via phage between phylogroups.

Many T3Es are mobilized between bacteria on GIs. GIs were identified for the three PacBio-sequenced strains of Psm R1, Psm R2 and Ps (Tables S15–S17). R1-5244 GIs contained the coro-

Table 3 Order of effectors in genomes sequenced using PacBio/MinION methods

| Contig    | Length | Effector                                                                 |
|-----------|--------|--------------------------------------------------------------------------|
| R1-5244   |        |                                                                          |
| Chromosome|        | hopAZ1, hopA2*, avrE1, hopM1*, hopAA1*, hopA2*, hopZ4, hopAT1, hopQ1, hopD1, hopR1, hopF4, hopBL2, hopV1, hopAR1, hopBK1* |
| Plasmid   |        | hopAF1-1, hopBF1, avrD1, avRpm2, hopBD1                                   |
| Plasmid   |        | hopA1                                                                    |
| Plasmid   |        |                                                                          |
| R1-5300   |        |                                                                          |
| Chromosome|        | hopV1, hopAZ1, avrA1, hopQ1-2, hopA2*, avrE1, hopM1*, hopAA1*, hopAB1, hopQ1, hopD1, hopR1, hopAO2*, avRpm2, avPto1, hopAS1, hopAT1*, hopBL2*, hopI1, hopAE1, hopAF1-2, hopF3, hopAY1*, hopAU1, hopAH1 |
| Plasmid   |        | hopW1, hopBK1*, hopAR1                                                  |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| Plasmid   |        |                                                                          |
| R2-leaf   |        |                                                                          |
| Chromosome|        | hopY1, hopAS1, hopAT1, hopH1, hopF4, hopW1, hopR1, hopAG1*, hopAH1-2, hopA1, hopN1, hopAA1*, hopMT, avrE1*, hopF2, hopE1, hopA2, hopAH1-1, hopAH1-1, hopAB3*, avrRps4, hopS2, hopI1, hopAR1 |
| Plasmid   |        | hopAO1*, hopAZ1, hopAY1                                                 |
| Plasmid   |        | hopD1*, hopAU1                                                          |
| Plasmid   |        | hopAF1-1, hopBF1                                                        |
| Plasmid   |        | hopBB1, hopBD1                                                          |
| Plasmid   |        | avrB2, hopX1                                                            |
| syr9097   |        |                                                                          |
| Chromosome|        | hopAG1, hopAH1, hopA11, avrRpm1, hopAR1, hopI1, hopAE1, hopBE1, hopAF1, hopAH1, hopAW1*, hopH1, hopA2, avrE1, hopM1, hopAA1* |

Effectors are listed in order of appearance on each assembly contig (labelled as chromosomal or plasmid). Where effectors could be considered as linked (within 10 kb of each other) they are underlined.

*Effector gene is disrupted and is labelled as a pseudogene.

†Effectors within the conserved effector locus.
found on genomic islands. These GIs were then searched for in other *P. syringae* genomes to identify potential sources of transfer, and Fig. S20 shows heatmaps of GI presence. The *Psm* R1 GIs included several found only in pathogenic *Psm* R1 strains, differentiating them from the nonpathogens. These included the corontative biosynthesis cluster (G11), *hopF3* (G16) and *hopAT1* (G14). Most *Psm* R1 GIs produced hits across *P. syringae*, particularly in P1 and P3. *Psm* R2 GIs were most commonly found in P1. Several were shared with other cherry-pathogenic clades, including those containing *hopAF1* (G136), *hopAT1* (G13) and *hopD1* (G16). Finally, although most islands identified in *syr9097* were commonly found across the species complex, those containing T3Es (G130, G123 and G126) appeared to be P2 specific, indicating that cherry-pathogenic strains likely gained these islands from other members of P2.

**Functional analysis of potential *avr* genes**

To validate predictions from genome analysis, cloning was used to identify avirulence factors active in cherry. The effector genes *avrPto* and *hopAB* were absent from cherry pathogens, and their evolution was theoretically linked to pathogenicity. Several other candidate avirulence effectors were identified that were absent from cherry pathogens but present in close out-groups (Fig. 6). Avirulence-gene identification focused on *Psm* R1, as any T3E variation within this clade may be due to differences in host specificity rather than phylogenetic distance. Potential avirulence T3E genes included *avrA1*, *avrPto1*, *hopAA1*, *hopAB1*, *hopAO2* and *hopG1*, which had full-length homologues in nonpathogenic *Psm* R1 strains but were absent from or truncated in pathogens. These genes were cloned from the strain R1-5300 (except *hopAO2*, which was cloned from R1-9657).

The effector *avrPto4* was also cloned from *P. syringae pv avellaneae* (Psv) BPIC631, a close relative of *Psm* R2. This effector was absent from most cherry-pathogenic strains (Fig. 6). Several pathogens possessed the full-length gene (R2-leaf, R2-9095 and *P. syringae pv avii*), but lacked the KRYV domain that functions in planta (Fig. S21) (Sohn et al., 2009). The *hopAW1* gene was cloned from *P. syringae pv avii* as this T3E has undergone two independent mutations in Psv strains, disrupting the beginning of the gene (Fig. S22). Finally, *hopCl* was cloned from the *Aquilegia vulgaris* pathogen RMA1, which is basal to the *Psm* R2 clade as it is absent from all cherry-pathogenic strains.

Nine effectors were cloned into pBBR1MCS-5 and conjugated into three pathogenic strains (R1-5244, R2-leaf and sry9644). The presence of the plasmids did not affect multiplication in *vitro*. Knock-out strains for the T3SS gene *hrpP* were obtained for R1-5244 and R2-leaf to act as nonpathogenic controls that could not secrete T3Es and failed to cause the HR on tobacco (Fig. S23).

Bacterial multiplication experiments were conducted in cherry leaves. The transconjugants expressing HopAB1 or HopCl failed to multiply to the same levels as the pathogenic empty vector (EV) controls or produce disease lesions. The ectopic expression
of AvrA1, AvrRps4 and HopAW1 also caused significant reductions in growth, but this reduction was not consistently seen across all three pathogenic strains (Fig. 7a). As the addition of the hopAB1 gene reduced pathogenicity, full-length hopAB2 and hopAB3 genes were also cloned from PsvBPIC631 and RAM1, and were also found to reduce pathogen multiplication (Fig. 7b).

To investigate the induction of the HR by the HopAB family and HopC1, inoculations were performed at high concentrations (2 x 10⁸ colony-forming units (CFU) ml⁻¹) as in Hulin et al. (2018). In Psm R1 and R2, the addition of these T3Es led to more rapid tissue collapse than observed in EV controls, indicative of HR induction (Fig. 7c,d); HopC1 and HopAB1 were particularly effective. With Ps, however, EV transconjugants themselves caused rapid tissue collapse, making it impossible to recognize an induced HR as symptom development was not significantly different.

The hopAB1 gene is found in a mobile-element-rich c. 40 kb region in the nonpathogenic Psm R1-5300, missing from the pathogen Psm R1-5244 (Fig. 8a). Meanwhile, Psm R2 and P. syringae pv avii possessed putatively pseudogenized hopAB3 alleles (Fig. 8b), and P. syringae pv avii also possessed a truncated hopAB1 gene (Fig. S2A). hopAB3 is truncated in Psm R2 due to a 2 bp insertion (GG at position 1404 bp) leading to a premature stop codon, whilst in P. syringae pv avii a 218 bp deletion has disrupted the C-terminus. If expressed, the E3-ubiquitin ligase domain is completely absent from the Psm R2 protein and disrupted in P. syringae pv avii (Fig. 8c). Both HopAB3 alleles were also divergent enough that the Pto-interacting domain (PID) was not identified by Interproscan. To determine if the truncated Psm R2 HopAB3 allele induced any resistance response in cherry leaves, the gene was expressed in Psm R1-5244 and population growth measured. The addition of this gene did not lead to a significant reduction in growth compared with the EV control, unlike other hopAB alleles (Fig. 8d), and the transconjugant was still able to induce disease symptoms 10 d post inoculation (dpi) (Fig. 8e).

Overall, the data supported the conclusion that expressing alleles of hopAB and hopC reduced bacterial multiplication in cherry and were consistent with HR induction by Psm R1 and R2. However, it should be noted that any growth changes exhibited might have been influenced by aberrant transcription or translation of these effectors in the plant due to expression in trans.

### Discussion

#### Core-genome phylogenetics

Phylogenetic analysis confirmed that cherry pathogenicity has evolved multiple times within P. syringae Psm R1, R2 and P. syringae pv avii each formed distinct monophyletic clades, whereas cherry-pathogenic Ps strains were distributed across the P2 clade, indicating that cherry pathogenicity has either evolved multiple times within P2 or that this clade is not particularly specialized. To confirm this genomic prediction of pathogenicity, several additional P2 strains isolated from bean, pea and lilac were tested for pathogenicity in cherry. They each produced lower population levels in cherry leaves than cherry pathogens, suggesting that strains isolated from cherry and plum are more pathogenic to their hosts of origin (Fig. S10). Many P2 strains have previously been named Ps on the basis of lilac pathogenicity, despite being pathogenic to other plant species (Young, 1991). A new naming system within this phylogroup is desirable.

#### Search for candidate effectors involved in cherry pathogenicity

Gains and losses of T3Es were closely associated with pathogenicity. Virulence-associated effectors hopA1, hopB1, hopH1 and hopBF1 had been gained in multiple cherry-pathogenic clades. The hopA1 effector has been studied in the bean pathogen P. syringae pv phaseolicola R3 (1302A), as a GI-located avr gene (avrPphB) whose protein is detected by the corresponding R3 resistance protein in planta (Pitman et al., 2005; Neale et al., 2016). HopA1 also acts as a virulence factor as a cysteine protease which targets receptor-like kinases to interfere with plant

---

**Table 4** List of putative horizontal gene transfer events that have occurred between cherry-infecting clades within Pseudomonas syringae

| Effector | Putative transfers | Region | Plasmid location | Predicted in RANGER-DTL |
|----------|-------------------|--------|------------------|------------------------|
| avrD1    | R1/R2/P. syringae pv avii | Plasmid | tig3 (R1-5244) | Y |
| avrRps4* | R2/P. syringae pv avii | Next to cluster of mobile elements | – | Y |
| hopAF1   | R1/R2/P. syringae pv avii | Plasmid | tig3 (R1-5244), tig6 (R2) | Y |
| hopAO1*  | R1/R2/P. syringae pv avii | Plasmid | tig5(R2), tig84 (R1-5300) | N |
| hopAT1   | R1/R2 | Genomic island | – | N |
| hopAU1   | R2/P. syringae pv avii | Plasmid | tig4 (R2) | Y |
| hopAY1   | R2/P. syringae pv avii | Plasmid | tig5 (R2) | Y |
| hopBB1   | R1/R2/P. syringae pv avii | Plasmid | tig8 (R2), tig65 (R1-5300) | Y |
| hopBD1   | R2/P. syringae pv avii | Plasmid | tig3 (R1-5244), tig8 (R2) | Y |
| hopBF1   | R1/R2/P. syringae pv avii | Plasmid | tig3 (R1-5244), tig6 (R2) | Y |
| hopD1*   | R2/P. syringae pv avii | Plasmid | tig4(R2) | N |
| hopO1    | R2/P. syringae pv avii | Next to cluster of mobile elements (next to hopT1) | – | Y |
| hopT1    | R2/P. syringae pv avii | Next to cluster of mobile elements (next to hopO1) | – | Y |

Where the effector gene is present in the PacBio- or MinION-sequenced strains, its chromosomal or plasmid location is indicated. The type III effector genes hopO1 and hopT1 were not present in the PacBio-sequenced strains and therefore it is uncertain if they are on plasmids or chromosomal.

*Effector gene is disrupted in some strains and is labelled as a pseudogene.
PAMP-triggered immunity (PTI) responses (Zhang et al., 2010). This effector could play a similar role in PTI suppression in cherry.

HopBB1 and other members of the HopF family were abundant in cherry pathogens. All HopF members share an N-terminus and myristoylation sites for plant cell membrane localization (Lo et al., 2016) and interfere with PTI and ETI in model plants (Wang et al., 2010; Wu et al., 2011; Hurley et al., 2014). The presence of multiple hopF homologues in cherry pathogens and specific gain of hopBB1 suggested the importance of their function. In comparison, HopH1 and HopBF1 are understudied. HopH1 is a protease, homologous to the Ralstonia
solanacearum Rip36 protein (Nahar et al., 2014). This T3E gene was found on GI37 in Psm R2-leaf and was within 3 kb of hopF4 (Fig. S25), indicating that these two T3Es may have been gained together. HopBF1 was first discovered in P. syringae pv apiata and oryzae (Baltrus et al., 2011), but its role is undetermined. This study therefore identified candidate T3Es important for cherry pathogenicity that should be the focus of future functional studies.

Fig. 5 Evolution of hopAR1 in different clades of Pseudomonas syringae containing cherry pathogens. (a) Maximum likelihood phylogenetic tree built using the nucleotide sequences of the hopAR1 gene. Cherry and plum isolated strains are highlighted in pink and blue respectively; those names followed by single asterisks were nonpathogenic on cherry in controlled pathogenicity tests. Strains with long-read sequenced genomes are in black boxes. Bootstrap supports < 99% are shown. The bar is nucleotide substitutions per site. Double asterisks point to the clustering of P. syringae pv morsprunorum (Psm) R2 sequences with syr2675. (b) Genomic locations of the hopAR1 gene in the three PacBio-sequenced cherry pathogens. The gene is located within prophage sequences in Psm R1 and R2 (see Table S14 for details), whereas in syr9097 it is on a genomic island (GI) adjacent to a transfer RNA (tRNA) gene. Effector genes are coloured in red, other coding sequences in blue, phage genes predicted by PHASTER and mobile element genes are in green, tRNA genes in pink and GIs predicted (GI14 in Psm R2 and GI23 in P. syringae pv syringae (Pss)) in light blue. Predicted phage att sites are in dark green, with sites homologous to R2-leaf in Pss 9097 also indicated even though a phage is not predicted here. The ends of predicted prophage sequences are denoted with dashed green lines. *hopBK1 is a pseudogene in this strain. CDS, coding sequence; ME, mobile element; T3E, type III effector.
Phytotoxin biosynthesis gene clusters were also identified. Coronatine is present on a plasmid in pathogenic \textit{Psm} R1 and may be one of the factors that differentiate pathogens from non-pathogens in this clade. Coronatine functions in virulence by downregulating salicylic acid defence signalling (Grant & Jones, 2009). Necrosis-inducing lipodepsipeptide toxins were common in P2. All cherry-pathogenic \textit{Pss} strains possessed at least one biosynthesis cluster. The ability of \textit{Pss} strains to cause necrosis on cherry fruits has been linked to toxins (Scholz-Schroeder et al., 2001). Interestingly, two nonpathogenic P2b cherry strains lacked all phytotoxins, a deficiency that probably contributes to their lack of pathogenicity.

All cherry-pathogenic \textit{Pss} strains had reduced effector repertoires. This observation supports the hypothesis that a phenotypic trade-off exists, with strains retaining few T3Es, whilst relying more on phytotoxins for pathogenicity (Baltrus et al., 2011; Hockett et al., 2014). If this pathogenic strategy has evolved in the P2 clade, it raises the question as to how it affects host specificity and virulence. P2 strains often infect more than one host species (Rezaei & Taghavi, 2014). These strains probably possess fewer ETI-inducing avirulence factors that restrict effector-rich strains to particular hosts, so may be more successful generalists. The reduction in T3E repertoire, however, may be limiting, as strains may be less capable of the long-term disease suppression required at the start of a hemi-biotrophic interaction.

Most cherry-pathogenic clades possessed genes involved in aromatic compound degradation, shown to be important in virulence on olive (Caballo-Ponce \textit{et al.}, 2016), and ice nucleation genes that stimulate frost damage (Lamichhane \textit{et al.}, 2014). The fact that not all cherry-pathogenic clades possessed these genes suggests they are not essential requirements for bacterial canker; however, they may contribute to niche persistence. For example, Crosse & Garrett (1966) observed that \textit{Psm} R1 survived in cankers for longer than \textit{Ps}. Increased persistence might be linked to genes involved in woody-tissue adaptation.

Horizontal gene transfer has been important in the acquisition of key effectors

HGT is important for effector shuffling within \textit{P. syringae} (Arnold & Jackson, 2011). Pathogenicity-associated T3Es \textit{hopBB1} and \textit{hopBF1} were plasmid encoded and showed evidence of HGT between the cherry-pathogenic clades in P1 and P3. Plasmid profiling revealed that cherry pathogens in these phylogroups possessed native plasmids, some of which were putatively conjugative, indicating the importance of plasmids in gene exchange. By contrast, most cherry-pathogenic \textit{Pss} strains lacked plasmids.

The T3E \textit{hopAR1} was chromosomal in all long-read sequenced genomes. This gene was found within distinct prophage sequences in \textit{Psm} R1 and R2. To our knowledge this is the first reported example of a plant pathogen T3E located within a prophage sequence. Interestingly, the \textit{Psm} R2 \textit{hopAR1} gene homologue was most similar to \textit{hopAR1} from a P2 bean strain \textit{syf2675}, which is a close relative of cherry \textit{Ps}. This strain possessed a homologous phage to \textit{Psm} R2, indicating that HGT of this T3E between phylogroups may have been phage mediated. This striking example of convergent acquisition of \textit{hopAR1} in the cherry pathogens, putatively through distinct prophages in \textit{Psm} R1 and R2, and a GI in \textit{Ps} indicates that this T3E may have important roles in virulence. The well-characterized
Fig. 7 Identification of avirulence factors activating effector-triggered immunity in cherry. (a) Boxplot of an initial 10-d population count analysis of cherry pathogens (R1-5244, R2-leaf and syr9644) transconjugants expressing candidate avirulence genes. The data presented are based on one experiment, with three leaf replicates and three nested technical replicates ($n = 9$). Boxplots show median and interquartile range (IQR) and whiskers extend to values 1.5 × IQR above and below the median. All data points are plotted with circles. Controls included the wild-type strain, a strain containing the empty pBBR1MCS-5 vector and a ΔhrpA deletion mutant (for R1-5244 and R2-leaf). A separate ANOVA was performed for each cherry pathogen (R1-5244, R2-leaf and syr9644) and the Tukey-HSD significance groups ($P = 0.05$; confidence level: 0.95) for each strain are presented above each boxplot. (b) Boxplot of 10-d population counts of cherry pathogens (R1-5244, R2-leaf and syr9644) expressing different HopAB alleles and HopC1. The data presented are based on three independent experiments ($n = 27$). Tukey-HSD significance groups are presented above each boxplot. (c) Symptom development of R1-5244, R2-leaf, syr9644 transconjugants. Mean symptom score values are presented and represent two independent experiments ($n = 6$). Symptoms assessed as degree of browning of the infiltration site: 1, limited browning; 2, <50%; 3, >50%; 4, 100% of the infiltrated area brown. Analysis was based on area under disease progress curve (AUDPC) values (0–48 h). An ANOVA was performed on AUDPC values, with asterisks indicating significantly different from the empty vector (EV) control. (d) Symptom development over time on a representative leaf inoculated with R1-5244 transconjugants. HPI, hours post inoculation. The order of strains: 1, EV; 2, hopAB1; 3, hopAB2; 4, hopAB3; 5, hopC1. Arrows indicate the first appearance of symptoms associated with each strain and are coloured based on the graph in (c). ANOVA tables for all statistical analyses are presented in Tables S18–S24, and AUDPC values are in Table S25. CFU, colony-forming units.
P. syringae pv phaseolicola R3 homologue is not associated with a phage, but has been shown to undergo dynamic evolution on a mobile genomic island in planta in resistant bean cultivars (Neale et al., 2016).

Several T3Es in Psm R1, R2 and Ps were located on GIs. To determine the likely source of GIs in cherry strains, all other P. syringae strains were searched for homologous sequences. There was evidence of Psm R1 and R2 islands being shared between cherry pathogen clades indicative of HGT events occurring between strains occupying the same ecological niche.

Functional genomics revealed convergent loss of an avr factor

Genes from the hopAB and avrPto families form a redundant effector group (REG) vital for early PTI suppression in herbaceous species (Jackson et al., 1999; Lin & Martin, 2005; Kvitko et al., 2009). Both effectors also trigger ETI by interacting with the serine-threonine kinase R protein Pto in tomato (Kim et al., 2002).

Across the P. syringae complex, the REG was common (Fig. S26), but cherry pathogens all lacked full-length genes. The hopAB1 gene has been lost from Psm R1, whilst the Psm R2 and P. syringae pv avii predicted HopAB3 proteins lacked the PID and E3-ubiquitin ligase domains through contrasting mutations. P. syringae pv avii also possessed a truncated hopAB1 gene (Fig. S24), lacking the PID domain. The lack of a PID in cherry pathogen HopAB proteins suggested that they could have diverged to avoid a Pto-based recognition system in cherry.

Full-length members of this REG were expressed in cherry pathogens to determine their role in planta. The addition of HopAB alleles (HopAB1–3) consistently reduced population growth of pathogenic strains in planta and triggered a response consistent with the HR. If this effector does trigger immunity in cherry, there may have been selection pressure for its loss or pseudogenization in cherry pathogens in order to reduce avirulence activity. The truncated version of HopAB3 in R2-leaf was found not to exhibit avirulence activity as its expression did not reduce growth of pathogenic strains in planta. Although AvrPto is part of the same REG, its expression had no effect on the ability of cherry pathogens to multiply in leaves. The absence of AvrPto from cherry pathogens is therefore unlikely to be driven by avirulence, but could be due to the lack of HopAB virulence targets in planta.

Fig. 8 hopAB alleles have been both lost and truncated in cherry pathogens. (a) Alignment of the DNA region surrounding hopAB1 in Pseudomonas syringae pv morsprunorum (Psm) R1 strains. Grey indicates sequence identity, whereas black indicates divergence. The effector genes are coloured in red, whereas other coding sequences are in blue and mobile element genes are in green. Asterisks indicate the location of hopAB1 in R1-5300, whilst the upstream effectors are hopQ1, hopD1 and hopR1. (b) DNA alignment of the hopAB3 gene of Psm R2 and close out-groups. Asterisks indicate where the hopAB3 gene has been truncated due to a GG insertion at 1404 bp leading to a frameshift in Psm R2, whilst in P. syringae pv avii (avii3846) there is a deletion at the end of the gene. (c) Diagrams showing the location of key domains in the HopAB3 protein including the Pto-interaction domain (PID), BAK1-interacting domain (BAK1) and E3 ubiquitin ligase (E3). The E3 domain is lost completely from the Psm R2 protein, whereas in avii3846 the beginning of this domain is lost. The PID domain was not detected in the cherry pathogen sequences. (d) Boxplot of 10-d population counts of R1-5244 transconjugants expressing three different full-length hopAB alleles, truncated hopAB3R2-leaf and hopC1. The boxplots were constructed as in Fig. 7. The data presented are based on two independent experiments (n = 18). Tukey-HSD significance groups (P = 0.05; confidence level: 0.95) are presented above each boxplot (full statistical analysis is in Table S26). (e) Representative image of symptoms 10 d post inoculation (dpi) with the different R1-5244 transconjugants when inoculated at a low concentration (2 × 10^6 colony-forming units (CFU) ml^{-1}) to observe pathogenicity. Arrows point to pathogenic symptoms in the strain expressing hopAB3R2-leaf and the empty vector (EV) strain, colour coded as in (d).
As this REG is vital for early disease suppression in model strains, cherry pathogens must rely on other T3Es to fulfil this role.

The variation in hopAB1 presence in Psm R1 is intriguing. Psm R1 strains may be pathogenic on both cherry and plum (D hopAB1) or just pathogenic on plum (possessed hopAB1) as recorded in Hulin et al. (2018). This suggests that the host proteins in cherry that detect the presence of HopAB are not present/functioning in plum. Future studies may determine how the two host immune responses diverged and could examine hopAB diversity across Prunus pathogens. This study focused on bacterial canker of P. avium; however, strains isolated from additional Prunus spp. that cause other diseases were included, such as P. syringae pv cerasi (bacterial gall of hybrid cherry Prunus × yedoensis, Kamiunten et al., 2000), P. syringae pv morsprunorum FTRSU7805 (canker of apricot), P. syringae pv amygdali (canker of almond) and P. syringae pv persicae (decline and canker of peach) (Table 1). All apart from P. syringae pv amygdali 3205 and P. syringae pv persicae lacked HopAB (Fig. 2), indicating that there may be a conserved resistance mechanism regulating ETI activated by this effector family in Prunus species.

Linking genomics to the evolution of cherry pathogenicity

Cherry pathogenicity has arisen independently within P. syringae, with strains using both shared and distinctive virulence strategies. Cherry-pathogenic clades in P1 and P3 have large effector repertoires. Cherry Pss were found across P2 with reduced T3Es and several phytotoxin gene clusters. Key events in the evolution of cherry pathogenicity (Fig. 9) appear to be the acquisition of virulence-associated effectors, often through HGT. Putatively important T3Es included hopAR1, members of the hopF family such as hopBB1 and the other T3Es hopBF1 and hopH1. Significantly, the loss/pseudogenization of HopAB effectors has also occurred in multiple clades. Within P2, the different cherry-infecting Pss clades have slight differences in their virulence factor repertoires that may reflect their convergent gain of pathogenicity. Clades differed in T3E content, phytotoxin genes and possession of genes for catechol degradation (Fig. 2), and thus pathogenicity was achieved with variable virulence factor repertoires. This study demonstrates that populations genomics can be used to examine a complex disease of a perennial plant species. A huge dataset was
narrowed down to several candidate host-specificity-associated genes, two of which (hopAB and hopCI) encode proteins that had putative avirulence functions in planta.

Acknowledgements

We acknowledge the East Malling Trust, University of Reading and BBSRC for funding (BB/P006272/1). Library preparation for PacBio sequencing was performed at the Earlham Institute, whilst for wild cherry strains Illumina MiSeq sequencing, libraries were prepared at the Genomics Facility, University of Warwick. We thank Steve Roberts, Helen Neale, Mateo San José and David Gutman for providing bacterial strains. We thank the East Malling Farm and Glass staff for plant maintenance. The authors declare no conflict of interest.

Author contributions

M.T.H., J.W.M., R.W.J. and R.J.H. conceived and designed the study as well as writing the manuscript. M.T.H. performed bioinformatics, statistical analysis and laboratory work. J.G.V. isolated some of the strains used in this study, J.G.V. and E.B.H. selected representative strains and prepared DNA for MiSeq sequencing of nine strains and L.B. assembled these nine sequences. H.J.B. performed the MinION library preparation and sequencing. A.D.A. assisted in bioinformatics pipeline development. All authors read and reviewed the final manuscript.

ORCID

Richard J. Harrison http://orcid.org/0000-0002-3307-3519

References

Alfano JR, Charkowski A, Deng W, Badel J, Petnicki-Ocwieja T, van Dijk K, Collmer A. 2000. The Pseudomonas syringae Hrp pathogenicity island has a tripartite mosaic structure composed of a cluster of type III secretion genes bounded by exchangeable effector and conserved effector loci that contribute to parasitic fitness and pathogenicity in plants. Proceedings of the National Academy of Sciences, USA 97: 4856–4861.

Almeida NF, Yan S, Lindeberg M, Studholme DJ, Schneider DJ, Condon B, Liu H, Viana CJ, Warren A, Evans C et al. 2009. A draft genome sequence of Pseudomonas syringae pv. tomato DC3000. Molecular Plant-Microbe Interactions: MPMI 22: 52–62.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.

Arnold DL, Jackson RW, Wood JR, Brown J, Mansfield JW. 2001. Molecular characterization of atrrPPhd, a widely-distributed gene from Pseudomonas syringae pv. phaseolicola involved in non-host recognition by pea (Pisum sativum). Physiological and Molecular Plant Pathology 58: 55–62.

Arnold DL, Jackson RW. 2011. Bacterial genomes: evolution of pathogenicity. Current Opinion in Plant Biology 14: 385–391.

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsmka K, Gerdes S, Glass EM, Kubal M et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9: 75.

Baltrus DA, Dougherty K, Beckstrom-Sternberg SM, Beckstrom-Sternberg JS, Foster JT. 2014a. Incongruence between multi-locus sequence analysis (MLSA) and whole-genome-based phylogenies: Pseudomonas syringae pathovar pisici as a cautionary tale. Molecular Plant Pathology 15: 461–465.

Baltrus DA, McCann HC, Gutman DS. 2017. Evolution, genomics and epidemiology of Pseudomonas syringae. Molecular Plant Pathology 18: 152–168.

Baltrus DA, Nishimura MT, Dougherty KM, Biswas S, Mukhtar MS, Vicente JG, Holub EB, Dangl JL. 2012. The molecular basis of host specialization in bean pathovars of Pseudomonas syringae. Molecular Plant–Microbe Interactions: MPMI 25: 877–888.

Baltrus DA, Nishimura MT, Romanchuk A, Chang JH, Mukhtar MS, Cherkis K, Roach J, Grant SR, Jones CD, Dangl JL. 2011. Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 Pseudomonas syringae isolates. PLoS Pathogens 7: 22.

Baltrus DA, Yourstone S, Lind A, Guillaud C, Sands DC, Jones CD, Morris CE, Dangl JL. 2014b. Draft genome sequences of a phylogenetically diverse suite of Pseudomonas syringae strains from multiple source populations. Genome Announcements 2: e01195-13.

Bansal MS, Alm EJ, Kellis M. 2012. Efficient algorithms for the reconciliation problem with gene duplication, horizontal transfer and loss. Bioinformatics 28: 283–291.

Bartoli C, Carrere S, Lamichhane R, Varvaro L, Morris CE. 2015a. Whole-genome sequencing of 10 Pseudomonas syringae strains representing different host range spectra. Genome Announcements 3: 1–3.

Bartoli C, Lamichhane JR, Berge O, Guillaud C, Varvaro L, Balestra GM, Vinatzer BA, Morris CE. 2015b. A framework to gauge the epidemic potential of plant pathogens in environmental reservoirs: the example of kiwifruit canker. Molecular Plant Pathology 16: 137–149.

Bender CL, Alarcon-Chaidez F, Gross DC. 1999. Pseudomonas syringae phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiology and Molecular Biology Reviews 63: 266–292.

Berge O, Monteil CL, Bartoli C, Chandysson C, Guillaud C, Sands DC, Morris CE. 2014. A user’s guide to a data base of the diversity of Pseudomonas syringae and its application to classifying strains in this phylogenetic complex. PLoS ONE 9: e105547.

Berlin K, Koren S, Chin C-S, Drake JP, Landolin JM, Philippy AM. 2015. Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. Nature Biotechnology 33: 623–630.

Bruns H, Cruisemak M, Letzel A-C, Alanjary M, McInerney JO, Jensen PR, Schulz S, Moore BS, Ziemert N. 2018. Efficient algorithms for the reconciliation problem with gene duplication, horizontal transfer and loss. Bioinformatics 34: 266–274.

Buonaurio R, Moretti C, da Silva DP, Cortese C, Ramos C, Venturi V. 2015. The molecular basis of host specialization in Pseudomonas syringae pv. tomato DC3000. Proceedings of the National Academy of Sciences, USA 110: 10181–10186.

Bull CT, de Boer SH, Denny TP, Fierro G, Fischer-Le Saux M, Saddinger GS, Scotichetini M, Stead DE, Takikawa Y. 2010. Comprehensive list of names of plant pathogenic bacteria, 1980–2007. Journal of Plant Pathology 92: 551–592.

Bulteux A, Kaluzna M. 2010. Bacterial cankers caused by Pseudomonas syringae on stone fruit species with special emphasis on the pathovars syringae and morrisonorum Race 1 and Race 2. Journal of Plant Pathology 92: 21–21.3.

Buonaurio R, Moretti C, da Silva DP, Cortese C, Ramos C, Venturi V. 2015. The olive knot disease as a model to study the role of interspecies bacterial communities in plant disease. Frontiers in Plant Science 6: 346.

Butler MI, Stockwell PA, Black MA, Day RC, Lamont IL, Poulter RTM. 2013. Pseudomonas syringae pv. actinidiarum from recent outbreaks of kiwifruit bacterial canker belong to different clones that originated in China. PLoS ONE 8:e57464.

Caballo-Ponce E, van Dillenwijn P, Wittich R, Ramos C. 2016. WHOP, a genomic region associated with woody hosts in the Pseudomonas syringae complex contributes to the virulence and fitness of Pseudomonas savastanoi pv. savastanoi in olive plants. Molecular Plant–Microbe Interactions 30: 113–126.
pathogenicity island, which contains genes for virulence and avirulence, on a large native plasmid in the bean pathogen *Pseudomonas syringae* pathovar *phaseolicola*. *Proceedings of the National Academy of Sciences, USA* 96: 10875–10880.

Joardar V, Lindeberg M, Jackson RW, Selengut J, Dodson R, Brinkac LM, Daugherty SC, Deboy R, Durkin AS, Giglio MG et al. 2005. Whole-genome sequence analysis of *Pseudomonas syringae* pv. *phaseolicola* 1448A reveals divergence among pathovars in genes involved in virulence and transposition. *Journal of Bacteriology* 187: 6488–6498.

Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* 444: 323–329.

Kamitunen H, Nakaol T, Oshida S. 2000. Agent of bacterial gall of cherry tree. *Journal of General Plant Pathology* 66: 219–224.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Kim YJ, Lin NC, Martin GB. 2002. Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity. *Cell* 109: 589–598.

Krivko BH, Collmer A. 2011. Construction of *Pseudomonas syringae* pv. *tomato* DC3000 mutant and polymutant strains. In: McDowell J, ed. *Plant immunity. Methods in molecular biology (methods and protocols)*, Vol. 712. New York, NY: USA: Humana Press.

Krivko BH, Park DH, Velasquez AC, Wei C-F, Russell AB, Martin GB, Schneider DJ, Collmer A. 2009. Deletions in the repertoire of *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion effector genes reveal functional overlap among effectors. *PLoS Pathogens* 5: e1000388.

Lamichhane JR, Varvaro L, Parisi L, Audergon J-M, Morris CE. 2014. Chapter four – Disease and frost damage of woody plants caused by *Pseudomonas syringae*. Seeing the forest for the trees. In: Sparks DL, ed. Advances in agronomy, Vol. 126. San Diego, CA: USA: Academic Press, 235–295.

Langmead B, Salzberg S. 2013. Fast gapped-read alignment with Bowtie2. *Nature Methods* 9: 357–359.

Larkin M, Blackshields G, Brown NP, Chenna R, Mcgettigan P, McWilliam K, Miller W, Eddy SR, Sadler M, Heger A, Ponting CP. 2007. Clustal W: a comprehensive approach for multiple sequence alignment. *Bioinformatics* 23: 2947–2948.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25: 2078–2079.

Li NC, Martin GB. 2005. *avrPtoB* of *Pseudomonas syringae* pv. *tomato* DC3000 is a functional effector. *PLoS ONE* 28: 467.

Lin NC, Martin GB. 2005. *avrPtoB* of *Pseudomonas syringae* pv. *tomato* DC3000 is a functional effector. *PLoS ONE* 28: 467.

Linn N, Martin GB. 2005. *avrPtoB* of *Pseudomonas syringae* pv. *tomato* DC3000 is a functional effector. *PLoS ONE* 28: 467.

Liskova A, Plucinska B,p, Dvorak A, Vojtisek J, Balazovjech M, Novakova M, Kutina L, Vejdosova L, Kolar V, Kolar M, et al. 2012. *Pseudomonas phaseolicola* and *Pseudomonas syringae* pv. *tomato DC3000* strains isolated from cherry fruit infected in the Czech Republic are molecularly identical. *New Phytologist* 195: 623–630.

Lombard V, Duret L, Michel Grondin S, Letunic I, Bork P. 2014. The InterProScan−InterProBLAST manual. *Briefings in Bioinformatics* 15: 42–46.

Lo T, Koulena N, Seto D, Guttman DS, Desveaux D. 2016. *Pseudomonas syringae* pv. *tomato* CRB2015: an atypical isolate with a large native plasmid in the bean pathogen *Pseudomonas syringae* pathovar *phaseolicola*. *Molecular Plant Pathology* 17: 458–469.

Lowe TM, Eddy SR. 1997. *Tatusov et al.* 2003. *Pseudomonas syringae* pv. *tomato DC3000* and its derivatives: comparison of genome sequences. *Proceedings of the National Academy of Sciences, USA* 105: 2025–2030.

Lu T, Koulina N, Seto D, Guttman DS, Desveaux D. 2016. The HopF family: an essential pathobiological effector family. *Molecular Plant Pathology* 17: 458–469.
Martínez-Garcia PM, Rodríguez-Palenzuela P, Arrebola E, Carrión VJ, Gutiérrez-Barranquero JA, Pérez-Garcia A, Ramos C, Cazorla FM, De Vicente A. 2015. Bioinformatics analysis of the complete genome sequence of the mango tree pathogen *Pseudomonas syringae* pv. *syringae* UMAP0158 reveals traits relevant to virulence and epiphytic lifestyle. *PLoS ONE* 10: 1–26.

Matsa IM, Castañeda-Ojeda MP, Aragon IM, Antúnez-Lamas M, Murillo J, Rodríguez-Palenzuela P, López-Solanailla E, Ramos C. 2014. Translocation and functional analysis of *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 type III secretion system effectors reveals two novel effector families of the *Pseudomonas syringae* complex. *Molecular Plant-Microbe Interactions* 27: 424–436.

Mazzaglia A, Studholme DJ, Taratufolo MC, Cai R, Almeida NF, Goodman T, Guttmann DS, Vinatzer BA, Balesta GM. 2012. *Pseudomonas syringae* pv. *actinidiae* isolates from recent bacterial canker of kiwifruit outbreaks belong to the same genetic lineage. *PLoS ONE* 7: 1–11.

McCann HC, Li L, Liu D, Pan H, Zhong C, Rikkerink EHA, Templeton MD, Straub C, Colombi E. 2017. Origin and evolution of the kiwifruit canker pandemic. *Genome Biology and Evolution* 9: 932–944.

McCann HC, Rikkerink EHA, Bertels F, Fiers M, Lu A, Rees-George J, van der Linden CE, Vinatzer BA. 2013. Genome-scale evolutionary inference identifies functions and clients of bacterial Hsp90. *PLoS Genetics* 9: e1003631.

Qi M, Wang D, Bradley CA, Zhao Y. 2011. Genome sequence analyses of *Pseudomonas savastanoi* pv. *glycines* and subtractive hybridization-based comparative genomics with nine pseudomonads. *PLoS ONE* 6: e16451.

Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: protein domains identifier. *Nucleic Acids Research* 33: 116–120.

Ravindran A, Jalan N, Yuan JS, Wang N, Gross DC. 2015. Comparative genomics of *Pseudomonas syringae* pv. *syringae* strains B301D and HS191 and insights into inzatpathovar traits associated with plant pathogenesis. *Microbiology Open* 4: 553–573.

Rezaei R, Taghavi SM. 2014. Host specificity, pathogenicity and the presence of virulence genes in Iranian strains of *Pseudomonas syringae* pv. *syringae* from different hosts. *Archives of Phytopathology and Plant Protection* 47: 2377–2391.

Rodríguez-Palenzuela P, Matas IM, Murillo J, López-Solanailla E, Bardaji L, Pérez-Martínez I, Rodriguez-Moskera ME, Penyalver R, López MM, Quesa JD et al. 2010. Annotation and overview of the *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 draft genome reveals the virulence gene complement of a tumour-inducing pathogen of woody hosts. *Environmental Microbiology* 12: 1604–1620.

Şahin F. 2001. Severe outbreak of bacterial speck, caused by *Pseudomonas syringae* pv. *tomato*, on field-grown tomatoes in the eastern Anatolia region of Turkey. *Plant Pathology* 50: 799.

Sawada H, Shimizu S, Miyoshi T, Shinozaki T, Kusumoto S, Noguchi M, Naridomi T, Kikuhara K, Kansako M, Fujikawa T et al. 2015. *Pseudomonas syringae* pv. *actinidiae* biovar 3. *Japanese Journal of Phytopathology* 81: 111–126.

Sawada H, Suzuki F, Matsuda I, Saitou N. 1999. Phylogenetic analysis of *Pseudomonas syringae* pathovars suggests the horizontal gene transfer of argK and the evolutionary stability of rhp gene cluster. *Journal of Molecular Evolution* 49: 627–644.

Sawyer S. 1989. Statistical tests for detecting gene conversion. *Molecular Biology and Evolution* 6: 526–538.

Scholz-Schroeder BK, Hutchison ML, Grgrunia I, Gross DC. 2001. The contribution of syringopeptin and syringomycin to virulence of *Pseudomonas syringae* pv. *syringae* strain B301D on the basis of *sypA* and *sypB* biosynthesis mutant analysis. *Molecular Plant-Microbe Interactions* 14: 336–348.

Schulze-Lefert P, Panstruga R. 2011. A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends in Plant Science* 16: 117–125.

Scortichini M. 2010. Epidemiology and predisposing factors of some major bacterial diseases of stone and nut fruit trees species. *Journal of Plant Pathology* 92: 73–78.

Sohn KH, Zhang Y, Jones JDG. 2009. The *Pseudomonas syringae* effector protein, AvrRP54, requires in planta processing and the KRVY domain to function. *Plant Journal* 57: 1079–1091.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Staskawicz BJ, Dahlbeck D, Keen NT. 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycines* determines race-specific incompatibility on *Glycine max* (L.) Merr. *Proceedings of the National Academy of Sciences, USA* 81: 6024–6028.
Thakur S, Weir BS, Gutmann DS. 2016. Phytopathogen genome announcement: draft genome sequences of 62 Pseudomonas syringae type and pathotype strains. *Molecular Plant–Microbe Interactions* 29: 243–246.

Vicente JG, Alves JP, Russell K, Roberts SJ. 2004. Identification and discrimination of *Pseudomonas syringae* isolates from wild cherry in England. *European Journal of Plant Pathology* 110: 337–351.

Visnovsky SB, Fiers M, Lu A, Panda P, Taylor R, Pitman AR. 2016. Draft genome sequences of 18 strains of *Pseudomonas* isolated from kiwifruit plants in New Zealand and overseas. *Genome Announcements* 4: e0061-16.

Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo MA, Zhao Y, Ma Z, Sundin GW. 2005.

Young JM. 2010.

Wu S, Lu D, Kabbage M, Wei H-L, Swingle B, Records AR, Dickman M, He F, Shan L. 2011.

Wang Y, Li J, Hou S, Wang X, Li Y, Ren D, Chen S, Tang X, Zhou J-M. 2010. *A Pseudomonas syringae* ADP-ribosyltransferase inhibits Arabidopsis mitogen-activated protein kinase kinases. *The Plant Cell* 22: 2033–2044.

Warnes G, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Macleider M, Magnusson A, Moeller S et al. 2016. *gplots*: various *R* programming tools for plotting data. [WWW document] URL: https://cran.r-project.org/web/packages/gplots/index.html [accessed 28 December 2017].

Wu S, Lu D, Kabbage M, Wei H-L, Swingle B, Records AR, Dickman M, He P, Shan L. 2011. Bacterial effector HopF2 suppresses Arabidopsis innate immunity at the plasma membrane. *Molecular Plant–Microbe Interactions* : 585–593.

Young JM. 1991. Pathogenicity and identification of the lilac pathogen, *Pseudomonas syringae* pv. *syringae* van Hall 1902. *Annals of Applied Biology* 118: 283–298.

Young JM. 2010. Taxonomy of *Pseudomonas syringae*. *Journal of Plant Pathology* 92: S1.5–S1.14.

Yu D, Yin Z, Li B, Jin Y, Ren H, Zhou J, Zhou W, Liang L, Yue J, Xu S. 2016. Gene flow, recombination, and positive selection in *Stenotrophomonas maltophilia*: mechanisms underlying the diversity of the widespread opportunist pathogen. *Genome* 59: 1063–1075.

Zhang J, Li W, Xiang T, Liu Z, Laluk K, Ding X, Zou Y, Gao M, Zhang X, Chen S et al. 2010. Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host & Microbe* 7: 290–301.

Zhao W, Jiang H, Tian Q, Hu J. 2015. Draft genome sequence of *Pseudomonas syringae* pv. *persicae* NCPPB 2254. *Genome Announcements* 3: e00555-15.

Zhao Y, Ma Z, Sundin GW. 2005. Comparative genomic analysis of the pPT23A plasmid family of *Pseudomonas syringae*. *Journal of Bacteriology* 187: 2113–2126.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Plasmid profiles of all *Prunus*-infecting sequenced strains and some out-groups for comparison.

**Fig. S2** Whole-genome alignment of R1-5244 and R1-5300 using ProgressiveMauve.

**Fig. S3** Initial core genome phylogeny of 108 *Pseudomonas syringae* strains.

**Fig. S4** Core genome phylogenetics of phylogroup 3 with removal of different out-group strains.

**Fig. S5** Core genome phylogenetics of phylogroup 2 with removal of different out-group strains.

**Fig. S6** Core genome phylogenetic tree with removal of the out-group strain *Pseudomonas syringae* pv *daphniphylli* 569.

**Fig. S7** Core genome phylogenetic tree with removal of the out-group strains *Pseudomonas syringae* pv *daphniphylli* 569 and *P. syringae* pv *syringae* 1212.

**Fig. S8** Core genome phylogenetic tree with removal of the out-group strain *Pseudomonas syringae* pv *eriobotryae* 4455.

**Fig. S9** Core genome phylogenetic tree with removal of the out-group strains *Pseudomonas syringae* pv *eriobotryae* 4455 and *P. syringae* pv *syringae* 1212.

**Fig. S10** Boxplot of 10-d population counts (Log CFU ml⁻¹) of *Pseudomonas syringae* pv *syringae* strains isolated from different host plant species, when inoculated into detached cherry leaves.

**Fig. S11** Organisation of the structural T3SS and conserved effector locus (CEL) of the three PacBio-sequenced pathogens (R2-leaf, sry9097 and R1-5244).

**Fig. S12** Alignment of the CEL in *Psm* R1.

**Fig. S13** Percentage of phylogenetic trees that supported the dependant model of evolution in the *BAYESTRATS* analysis for T3E families that were significantly associated with pathogenicity.

**Fig. S14** Predicted gain and loss of effector genes occurring on each branch leading to cherry pathogenic clades on the core genome phylogenetic tree.

**Fig. S15** Core genome phylogenetic tree used in Gloome gain and loss analysis with the branch labels added.

**Fig. S16** Maximum-likelihood phylogenetic trees for effector genes (*avrD-hopA1*) showing evidence of horizontal gene transfer between cherry pathogen clades.

**Fig. S17** Maximum-likelihood phylogenetic trees for effector genes (*hopBB-hopT*) showing evidence of horizontal gene transfer between cherry pathogen clades.

**Fig. S18** Core genome phylogenetic tree used in RANGER-DTL analysis with the branch labels added.

**Fig. S19** *hopA1* gene region in *Psm* R2 and phylogroup 2 strains.

**Fig. S20** Genomic islands characteristic of cherry pathogens are found across *Pseudomonas syringae*.

**Fig. S21** AvrRps4 alignment.

**Fig. S22** HopAW1 alignment.
Fig. S23 Representative images of the tobacco hypersensitive response assay for R1-5244 and R2-leaf ΔhrpA mutants.

Fig. S24 Protein alignment of members of the HopAB effector family.

Fig. S25 Genomic region containing the hopH1 gene in Psm R2-leaf.

Fig. S26 Heatmap showing the presence and absence of the avrPto/hopAB REG across the Pseudomonas syringae complex.

Table S1 All Pseudomonas syringae transconjugants and gene deletion mutants generated in this study

Table S2 All vectors/E. coli strains used in this study with antibiotic resistance information and reference

Table S3 All primers used in this study

Table S4 Protein sequences used in Blast (Altschul et al., 1990) analysis to identify virulence factors with NCBI accession number, gene name and abbreviation and source organism

Table S5 Plasmid-encoding gene identification in Psm R1-5244

Table S6 Plasmid-encoding gene identification in Psm R1-5300

Table S7 Plasmid-encoding gene identification in Psm R2-leaf

Table S8 Statistical information for different phylogenetic trees constructed from the core genome in this study

Table S9 Number of recombining genes in the core genome of each phylogroup estimated using GeneConv

Table S10 ANOVA table of 10 dpi population count of Pseudomonas syringae pv syringae outgroups on detached cherry leaves

Table S11 Effector presence comparisons between cherry pathogenic and nonpathogenic Psm R1 strains

Table S12 GLOOME output for all events occurring on branches leading to cherry pathogens and tips of the phylogeny

Table S13 Putative horizontal gene transfer events predicted using Ranger-DTL

Table S14 Output of Phaster identification of prophages in the three cherry pathogenic PacBio-sequenced strains

Table S15 Genomic islands predicted in Psm R1-5244

Table S16 Genomic islands predicted in Psm R2-leaf

Table S17 Genomic islands predicted in Pss 9097

Table S18 ANOVA table of 10 dpi population count of Psm R1-5244 transconjugants (all candidate avirulence genes) on detached cherry leaves

Table S19 ANOVA table of 10 dpi population count of Psm R2-leaf transconjugants (all candidate avirulence genes) on detached cherry leaves

Table S20 ANOVA table of 10 dpi population count of syr9644 transconjugants (all candidate avirulence genes) on detached cherry leaves

Table S21 ANOVA table of 10 dpi population count of Psm R1-5244 transconjugants of candidate avirulence genes hopAB alleles and hopC1 on detached cherry leaves

Table S22 ANOVA table of 10 dpi population count of Psm R2-leaf transconjugants of candidate avirulence genes hopAB alleles and hopC1 on detached cherry leaves

Table S23 ANOVA table of 10 dpi population count of syr9644 trans-conjugants of candidate avirulence genes hopAB alleles and hopC1 on detached cherry leaves

Table S24 ANOVA table of AUDPC symptom score analysis on detached cherry leaves

Table S25 AUDPC (Area Under the Disease Progress Curve) values for 0–48 h symptom development of pathogenic clades expressing different candidate avirulence genes

Table S26 ANOVA table of 10 dpi population count of Psm R1-5244 transconjugants of candidate avirulence genes hopAB alleles and hopC1 as well as the hopAB3 allele from cherry pathogen R2-leaf on detached cherry leaves

Methods S1 Detailed descriptions of methodology used.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the New Phytologist Central Office.

See also the Commentary on this article by Baltrus & Orth, 219: 482–484.