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1 **Phylogenetic evidence of the migration of turnip mosaic potyvirus from Europe to**  
2 **Australia and New Zealand**

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1 ABSTRACT

2

3 Thirty-two isolates of turnip mosaic virus (TuMV) were collected mostly from *Brassicaceae* plants in  
4 Australia and in New Zealand during 1994-2011. Host reaction studies showed that most of the isolates  
5 belonged to *Brassica* (B) host-infecting type. We mostly performed sequence-based phylogenetic and  
6 population genetic analyses of the complete genomic sequences and of three non-recombinogenic regions of  
7 those sequences (protein encoding regions of the partial helper-component proteinase protein, protein 3 and  
8 nuclear inclusion b protein). The substitution rates, divergence times, and phylogeographic patterns of the virus  
9 populations were estimated. Six inter- and five intralineage recombination type patterns were found in the  
10 genomes of the Australian and New Zealand isolates, and all were novel. Only one recombination type pattern  
11 has been found in both countries. Australian and New Zealand populations were genetically different, and  
12 were different from the European and Asian populations. Our Bayesian coalescent analyses, based on a  
13 combination of novel and published sequence data from three non-recombinogenic protein-encoding regions,  
14 showed that TuMV probably started to migrate from Europe to Australia and New Zealand more than 80  
15 years ago, and distinct populations arose as a result of evolutionary drivers such as recombination. The  
16 basal-B2 subpopulation in Australia and New Zealand seems to be older than those of the world-B2 and B3  
17 populations. Ours is the first population genetic study of TuMV in the Antipodes. It shows that the time of  
18 migration of TuMV correlates well with the establishment of agriculture and migration of Europeans to these  
19 countries.

20

1 INTRODUCTION

2 Studies of the genetic structure of populations of plant viruses are important for understanding virus  
3 evolution and emergence (García-Arenal *et al.*, 2001; Gibbs *et al.*, 2008; Gibbs & Ohshima, 2010), especially of  
4 those viruses that evolve at measurable rates and adapt rapidly to new or resistant hosts (Ohshima *et al.*,  
5 2010). These not only include plant viruses with RNA genomes but also those with single- and  
6 double-stranded DNA genomes, such as begomoviruses and mastreviruses in the family *Geminiviridae*  
7 (Lefeuvre *et al.*, 2010, Rocha *et al.*, 2013) and cauliflower mosaic virus in the family *Caulimoviridae* (Yasaka  
8 *et al.*, 2014). All of these reports have shown that the evolution of virus populations is shaped by founder effects,  
9 selection and recombination.

10 Potyviruses are RNA viruses and are among the most important pathogens of crops. They have spread  
11 throughout much of the subtropical and temperate zones of the world (Gibbs & Ohshima, 2010; King *et al.*,  
12 2012). Potato virus Y (Ogawa *et al.*, 2008; Visser *et al.*, 2012), turnip mosaic virus (TuMV) (Nguyen *et al.*, 2013b;  
13 Ohshima *et al.*, 2002), soybean mosaic virus (Seo *et al.*, 2009) and zucchini yellow mosaic virus (Lecoq *et al.*,  
14 2009) are important potyviruses with worldwide distributions. Nevertheless, there remains a poor  
15 understanding of how and when they dispersed, and of what factors controlled that spread

16 TuMV infects a wide range of plant species, most from the family *Brassicaceae* (Walsh & Jenner, 2002).  
17 TuMV, like other potyviruses, is transmitted by aphids in the non-persistent manner. TuMV has flexuous  
18 filamentous particles 700-750 nm long, each of which contains a single copy of the genome, which is a  
19 single-stranded, positive-sense RNA molecule of about 9,833 nucleotides. This is translated into one large  
20 polyprotein which hydrolyzes itself into at least 10 proteins (King *et al.*, 2012). Furthermore, an overlapping  
21 'pretty interesting *Potyviridae* ORF' (PIPO) exists in the +2 reading frame within the protein 3 (P3) encoding  
22 region (Chung *et al.*, 2008).

23 Earlier studies have shown that TuMV originated from wild orchids in Europe and then emerged to  
24 spread among species of *Brassicaceae* in the Mediterranean region, including Southeast Europe, Asia Minor  
25 and mid-Eurasia (Nguyen *et al.*, 2013b; Ohshima *et al.*, 2002; Tomimura *et al.*, 2004). Crops of the *Brassicaceae*  
26 that are most commonly cultivated in Europe are *Brassica* species, whereas both *Brassica* and *Raphanus* crops  
27 are important in Asia Minor and Asian countries (Nguyen *et al.*, 2013a; Tomimura *et al.*, 2003; Tomitaka &  
28 Ohshima, 2006; Tomitaka *et al.*, 2007). TuMV isolates are of five host-infecting types. OM-host type isolates  
29 infect some plants of *Brassicaceae* but not brassica plants, [(B)]-host type isolates infect *Brassica* plants latently  
30 and occasionally, but do not infect *Raphanus* plants, [B]-host type isolates infect most *Brassica* species  
31 systemically, causing mosaic of the systemically infected leaves, but do not infect *Raphanus* plants. [B(R)]-host  
32 type isolates infect most *Brassica* systemically, causing mosaic of systemically infected leaves, and infect  
33 *Raphanus* plants latently and occasionally. [BR]-host type isolates infect both *Brassica* and *Raphanus* plants  
34 systemically, causing mosaic of the systemically infected leaves. The basal-B cluster of (B) or B-host type  
35 isolates was most variable, was paraphyletic to the other lineages, and was isolated from both non- and  
36 *Brassicaceae* plants. The world-B cluster is the most variable and widespread cluster; most European isolates  
37 did not infect *Raphanus*, whereas Asian isolates infect both *Brassica* and *Raphanus*.

38 Turnip-mosaic was first reported in Australia and New Zealand in the 1930s (Chamberlain, 1936;  
39 Samuel, 1931) and characterized by symptoms, by host range and by sap- and aphid-transmission. More  
40 recent reports from Australia (Gibbs *et al.*, 2006; Schwinghamer *et al.*, 2014) and New Zealand (Fletcher *et al.*,  
41 2010; Ochoa Corona *et al.*, 2007) characterized the virus by molecular techniques and showed that two  
42 isolates from Australia were closely related to isolates from Europe. Here, we report an in-depth analysis of the  
43 populations of TuMV in Australia and New Zealand, mostly from *Brassicaceae* hosts, together with the full  
44 genomic sequences of 32 of the isolates. We use data from full and partial genomic sequences for evolutionary  
45 analyses, including recombination and phylogenetic analyses, and for the estimation of subpopulation

1 differentiation, relationships and divergence between their populations and those in Europe and Asia. We  
2 make phylodynamic comparisons using the genomic sequences of 229 isolates collected worldwide, and  
3 discuss what they reveal about the changes that have occurred during continent-wide evolution and migration  
4 of populations. Our analyses provide a preliminary definition of the present geographical structure of TuMV  
5 populations in Australia and New Zealand, and indicate that they reflect recent human immigration patterns  
6 and the agricultural history of the two countries.  
7

## 1 RESULTS

### 2 3 Biological and molecular characteristics

4  
5 A total of 32 TuMV isolates collected in Australia and New Zealand was examined in this study; 16 from  
6 eastern Australia, and one from the North Island and 15 from the South Island of New Zealand (Fig. 1, Table  
7 S1). Thus, isolates were collected from those parts of the two countries in which brassica crops are cultivated.  
8 One Australian isolate was from *Cicer arietinum*, a legume, and 15 from brassicas. The New Zealand samples  
9 included one from a crocus and 15 from brassicas and from the closely related plant, *Nasturtium officinale*. The  
10 viruses were found in commercial fields as well as in home gardens.

11  
12 All the Australian and New Zealand isolates infected *Brassica juncea* cv. Hakarashina and *B. rapa* cv.  
13 Hakatasuwari plants, but occasionally infected *B. oleracea* var. capitata cvs. Ryozan 2-go and Shinsei. They did  
14 not infect Japanese radish (*Raphanus sativus* cvs. Akimasari, Tailbyo-soubutori and Houryou). Therefore, we  
15 concluded that the Australian and New Zealand isolates are *Brassica* (B) host infecting type. Interestingly,  
16 Australian and New Zealand isolates showed local lesions on the inoculated leaves and then systemic  
17 symptoms in *Chenopodium quinoa*, whereas most Asian and European isolates showed local lesions only on  
18 the inoculated leaves (data not shown).

19  
20 We analysed the 32 full genomes sequenced in this study, as well as 197 full genomic TuMV sequences  
21 obtained from public DNA sequence databases. The 197 full genomic sequences contain the two published  
22 sequences from Australia and New Zealand. The genomes of 29 Australian and New Zealand isolates were 9,798  
23 nucleotides long excluding 5' end 35 nt primer sequences, whereas three New Zealand isolates (NZ403, NZ403B  
24 and NZ415) were two nucleotides shorter in the 3' non-coding region (NCR; 207 nt long). The regions encoding  
25 the protein 1 (P1), helper-component proteinase protein (HC-Pro), P3, PIPO, 6 kDa 1 protein (6K1), cylindrical  
26 inclusion protein (CI), 6 kDa 2 protein (6K2), genome linked viral protein (VPg), nuclear inclusion a-proteinase  
27 protein (NIa-Pro), nuclear inclusion b protein (NIb) and coat protein (CP) encoding regions were 1086, 1374,  
28 1065, 177, 156, 1932, 159, 576, 729, 1551 and 864 nucleotides long, respectively. All of the motifs reported for  
29 different potyvirus-encoded proteins were found. The new genomic sequences determined in this study have  
30 been deposited in DDBJ/EMBL/GenBank databases with Accession Codes AB989628–AB989659.

### 31 32 Genetic recombination in Australia and New Zealand

33  
34 The genomic sequences of 32 Australian and New Zealand isolates and 197 published sequences were  
35 assessed for evidence of recombination. Each of the identified sites was examined individually and a  
36 phylogenetic approach was used to verify the parent/donor assignments made using the RDP4 package  
37 (Martin *et al.*, 2010). Having examined all sites with an associated *P*-value of  $<10^{-6}$  (i.e., the most likely  
38 recombination sites), we retained the intralinear recombination sites (parents from the same major group lineage)  
39 and removed the interlineage recombination sites (i.e., those with parents from different major lineages) by treating  
40 the identified recombination sites as missing data in subsequent analyses.

41  
42 Twenty-one unequivocal recombination sites were found in the genomes of 34 Australian and New Zealand  
43 isolates (Fig. 2, Table S2). In the Australian population, one isolate (AUST21) was identified to be a  
44 non-recombinant of the world-B3 subgroup, whereas one isolate (BRS1) of the basal-B and two isolates  
45 (AUST19 and AUST23) of the world-B group were identified to be intralinear recombination sites. The other 13

1 isolates were interlineage recombinants between world-B and basal-B parents. In the New Zealand  
2 population, no non- and interlineage recombinants were found. All were intralinear recombinants of basal-B  
3 or world-B parents. Fourteen New Zealand isolates were single, double or triple intralinear recombinants of  
4 world-B parents. Triple intralinear recombinants of world-B parents (AUST19, AUST23 and NZ402) were  
5 present both in Australian and New Zealand populations but were not dominant in either country. Twenty  
6 recombination sites, except one at nt 6132 in the VPg encoding region, had not been found in other TuMV  
7 populations (Nguyen *et al.*, 2013b; Ohshima *et al.*, 2007), indicating that the Australian and New Zealand  
8 populations were distinct.

#### 9 10 Phylogenetic relationships

11  
12 A phylogenetic network was inferred using Neighbor-Net from the concatenated 5' NCR, main ORF and 3' NCR  
13 sequences (Fig. S1). Three isolates (AUST10, AUST13 and BRS1) from Australia, three isolates (NZ403,  
14 NZ403B and NZ415) from New Zealand, and many German, Italian and Spanish isolates of Europe  
15 recombinants and non-recombinants fell into the 'basal-B and recombinants' group. Furthermore, many  
16 Australian and New Zealand isolates with worldwide isolates fell into the 'world-B and recombinants' group  
17 and clustered with European isolates. None of Australian and New Zealand isolates (either full genomes or  
18 parts of them) grouped with the Orchis, 'basal-BR and recombinants', or 'Asian-BR and recombinants' groups.

19  
20 Because only one of the Australian and New Zealand isolates was not a recombinant, the relationships of the  
21 isolates were investigated using the three regions of the genomic sequences that gave the least evidence of  
22 recombination: Region A (nt 1460-3472, numbers corresponding to the positions in original UK 1 genome)  
23 covered part of the HC-Pro and P3 regions; Region B (nt 3812-6016) included part of the CI and VPg regions;  
24 and Region C (nt 6479-8068) included part of the NIa and NIb regions (see Fig. 2). Trees were calculated using  
25 225, 214 and 226 non- and intralinear recombinant sequences, respectively. The relationships of isolates  
26 were investigated by maximum likelihood (ML) implemented in PhyML (Guindon & Gascuel, 2003) (Fig. S2).  
27 These partitioned most of the sequences into the same five major consistent genetic groups, as reported  
28 previously (Nguyen *et al.* 2013b): Orchis, basal-B, basal-BR, Asian-BR and world-B. The basal-B group further  
29 split into basal-B1 and B2 subgroups and the world-B group split into the world-B1, B2 and B3 subgroups.

30  
31 In the Region A tree (Fig. S2a), 13 Australian isolates and three New Zealand isolates fell into the basal-B2  
32 subgroup, two Australian isolates and 13 New Zealand isolates fell into the world-B2 group, and only one  
33 Australian and one New Zealand isolate fell into the world-B3 group. In the Region B tree (Fig. S2b), two  
34 Australian isolates and three New Zealand isolates fell into the basal-B2 subgroup, three Australian isolates  
35 and 13 New Zealand isolates fell into the world-B2 group, and 12 Australian isolate fell into the world-B3  
36 subgroup. In the Region C tree (Fig. S2c), two Australian and three New Zealand isolates fell into the basal-B2  
37 subgroup, no Australian and nine New Zealand isolates fell into the world-B2 subgroup, and 14 Australian and  
38 five New Zealand isolates fell into the world-B3 group. These trees confirmed that none of the Australian or  
39 New Zealand isolates had regions from Orchis, basal-BR or Asian-BR parental lineages. For further sequence  
40 analyses, we used the parts of the HC-Pro, P3 and NIb encoding regions that contained no recombination  
41 cross-over points, in any sequence. We called these the HC-Pro\*, P3\* and NIb\* regions (see Methods).

#### 42 43 Genetic population structure

44  
45 The haplotype and nucleotide diversities of the Australian and New Zealand populations in the TuMV

1 phylogenetic groups were compared (Table S3). In most cases, haplotype diversity values were large and  
2 nucleotide diversity values were small (i.e., few single-nucleotide polymorphisms, but most of them unique). The  
3 nucleotide diversities of Australian isolates in most phylogenetic groups were greater than those of New Zealand  
4 isolates in the HC-Pro\* and P3\* regions; the two regions were similarly variable, but the N1b\* region less variable.  
5 Overall, the combination of high haplotype diversity and overall lack of nucleotide diversity within individual  
6 geographical groups indicate that there has been a recent population expansion. This was confirmed by the  
7 Bayesian molecular-clock analyses described below.

#### 8 9 Evolutionary rates and timescales

10  
11 A Bayesian phylogenetic method (Drummond *et al.*, 2012) was used to estimate the evolutionary rates and  
12 timescales for the HC-Pro\*, P3\* and N1b\* regions. The best-supported demographic models were of constant  
13 size for all protein-encoding regions (Table 1). A relaxed-clock model provided a better fit than the strict-clock  
14 model, indicating the presence of rate variation among lineages. The presence of an adequate temporal signal  
15 in the data was confirmed using a date-randomization test (Fig. S3), in which the calculated rate estimate was  
16 compared with estimates from date-randomized replicates. We note, however, that the date-randomization  
17 test involves the assumption of random phylogenetic and temporal sampling, which is unlikely to be met by our  
18 data set. The impact of non-random sampling on the test is unknown.

19  
20 The mean estimated substitution rates were  $1.47 \times 10^{-3}$  subs/site/year for HC-Pro\*,  $1.35 \times 10^{-3}$  subs/site/year  
21 for P3\* and  $1.30 \times 10^{-3}$  subs/site/year for N1b\* regions (Table 1). Mean estimates of the age of the root of all  
22 the TuMVs were 610 years for HC-Pro\*, 806 years for P3\*, and 679 years for N1b\* regions (Table 1, Fig. 3, Fig.  
23 S4). The relationships between Australian and New Zealand isolates and European country isolates were also  
24 confirmed using ML trees (Fig. S5). These estimates are potentially inflated by the inclusion of transient  
25 polymorphisms that would normally be removed by purifying selection over longer timeframes (Duchêne *et al.*,  
26 2014; Gibbs *et al.*, 2010; Wertheim & Kosakovsky Pond, 2011).

#### 27 28 Dating of recombination events

29  
30 We estimated the ages of recombination events (Table 2, Fig. S6) using the method described by Visser *et al.*  
31 (2012). Recombinant sequences were split into their separate regions and realigned using gaps. When a  
32 sequence is a recombinant with two 'parents', it is split into two regions and the empty sites are filled with gaps.  
33 In this way, one recombinant sequence becomes two non-recombinant sequences, each with missing data. The  
34 analysis of the split sequences indicated that the interlineage recombination sites of Australian isolates with  
35 basal-B2 and world-B3 parents at nt 818 and nt 3475 of Australian isolates occurred 50-10 and 51-22 years ago,  
36 and the intralinear recombination sites of basal-B2 parents nt 6019 of New Zealand isolates occurred 75-19  
37 years ago. The intralinear recombination sites of world-B parents at nt 5602 and nt 5665 of New Zealand  
38 isolates occurred 49-20 and 22-11 years ago. Therefore, the ages of basal-B parent related recombination  
39 events were older.

#### 40 41 Plausible routes of TuMV migration into Australia and New Zealand

42  
43 We investigated the likely routes of TuMV migration into Australia and New Zealand using a Bayesian  
44 phylogeographic analysis (Lemey *et al.*, 2009) of the HC-Pro\*, P3\* and N1b\* datasets of non-recombinant  
45 isolates. Isolates were tagged with their countries of provenance. Our results indicate that TuMV migrated



1 between European countries and from European countries to Australia and to New Zealand. Therefore, we  
2 further investigated the routes of migration for each phylogenetic subgroup; focusing on the basal-B2,  
3 world-B2 and world-B3 subgroups because Australian and New Zealand isolates were only from these three  
4 subgroups (Fig. 4). For instance, migrations from Germany to Australia and to New Zealand were supported  
5 by results from the HC-Pro\* and P3\* regions [Bayes factor (BF)=54 and BF=22 for HC-Pro\*, BF=129 and  
6 BF=63 for P3\*, respectively] and from Germany to Australia was supported by N1b\* region (BF=55) for  
7 basal-B2 subgroup (Fig. 4a). The estimated ages of migrations were about 83-70 [95% highest posterior  
8 density interval (HPD): 159-23] years ago for Australia and 45-32 (95% HPD: 72-16) years ago for New  
9 Zealand. The migration from Australia to New Zealand was supported only by N1b\* region (BF=68) and it was  
10 33 (95% HPD: 54-20) years ago. In contrast, the migrations of the world-B2 and world-B3 subgroups  
11 occurred more recently and these were within 42-20 (95%HPD; 63-12) years before present (Fig. 4b, c and d).  
12 There was also significant support (BF $\geq$ 100) for migration between the neighbouring countries within Europe  
13 and East Asia (Fig. S7).

14 This analysis was confirmed by ML trees of the HC-Pro\*, P3\* and N1b\* regions Fig S5. These show which  
15 isolates were closest to the Australian and New Zealand clusters, and were therefore likely to be from the  
16 populations that provided the migrants. For example, the ML analysis found that all the basal-B2 gene  
17 populations from Australia and New Zealand probably came from Germany (Fig. 4, Fig. S5), and it can be seen  
18 in the trees that the closest isolates were, for Australian populations, two from Germany, DEU 7 and A11A, and  
19 one from the USA (PV134), and for the New Zealand isolates, two from Germany, T1GA and T1GD, with many  
20 European isolates in sister clusters. Similarly the more recent world-B2 and -B3 migrants were, on average,  
21 closest to UK isolates (Fig. S5).

22

## 23 DISCUSSION

24

25 We present here a preliminary assessment of the genetic structure of the TuMV populations of Australia and  
26 New Zealand. Previously published data, including five CP gene sequences and one genomic sequence, indicated  
27 that there were at least two distinct lineages of TuMV in Australia (Gibbs *et al.*, 2006) and one in New Zealand,  
28 and that all were closely related to TuMVs found in Europe. Here we have reported the genomic sequences of  
29 34 isolates from Australia and New Zealand, allowing us to assess their position in the world TuMV population in  
30 both space and time. We used both Bayesian and ML methods to analyse dated isolates of known provenance.  
31 The two approaches gave closely similar and internally consistent results, which we have used to determine  
32 when, and from where, the present Australian and New Zealand populations of TuMV came. This also allowed  
33 us to evaluate whether they arrived by natural means or with human assistance.

34 Many of the isolates from Australia and New Zealand are recombinants, but all of the recombination  
35 cross-over points found in these isolates are in genomic positions that are clearly different from those in all  
36 isolates (195 isolates) from other parts of the world and known to us. This fact, together with estimates of the  
37 dates for the recombination events and for the divergence of the Australian and New Zealand isolates from their  
38 nearest relatives, shows that migration probably preceded recombination.

39 The Australian and New Zealand populations of TuMV are closely related to viruses found in Germany  
40 and the UK, which, in turn, are related to older and more diverse European populations. This indicates that the  
41 Australian populations are recent migrants from Germany and the UK. Fig. 4 summarizes the sources and likely  
42 migration dates of three genomic regions of the lineages that have been found. The clearest evidence is from the  
43 basal-B2 populations. These were the first to arrive in the Australia (from Germany, about 70 years ago) and  
44 New Zealand (from Germany about 35 years ago, its N1b gene via Australia). The isolates of the other two TuMV  
45 taxonomic groups, world-B2 and world-B3, arrived from the UK about 35 years ago. Thus, there is evidence that

1 a minimum of three TuMV isolates evaded quarantine and entered Australia, and four entered New Zealand  
2 Turnip mosaic disease was first recorded in both countries in the 1930s (Chamberlain, 1936; Samuel, 1931).  
3 This is consistent with the dates that we have obtained in our analyses of the Australian population, but may  
4 indicate that the earlier New Zealand population might not have been sampled, or might have not survived.

5 Our evidence indicates that TuMV is most likely to have migrated to Australia and New Zealand in plant  
6 materials imported from Europe (16-18,000 km), rather than from South East Asia (5-9,000 km) in imports, or  
7 by natural means such as flying aphids. This is because the known TuMV populations of the south and east of  
8 Asia, Japan, Vietnam and China, are genetically linked but distinct from those of Europe (Nguyen *et al.*, 2013ab;  
9 Tomimura *et al.*, 2003). Australia and New Zealand were first populated around 50,000 and 800 years ago,  
10 respectively, but regular trade to these countries, and the development of agriculture, did not start until about  
11 600 years ago and was mostly derived from Europe. These incursions have grown in volume ever since,  
12 especially since World War II (Wace, 1985). Although human migration to Australia and New Zealand was, until  
13 recently, dominated by people from the UK, an equal number came from mainland Europe. Hence, the UK and  
14 Europe have been important sources of crop seeds and weeds (Wace 1985, Zubareva *et al.*, 2013). As a  
15 consequence, it is not surprising that the TuMV populations of Australia and New Zealand are most closely  
16 related to those of Germany and the UK.

17 Interestingly the basal-B2 isolates from Australia and New Zealand are closest to German isolates from  
18 *Alliaria officinalis* (ALLA), *Lactuca sativa* (DEU7) and *Tigridia* spp. (TIGA and TIGD). All of these have  
19 horticultural, rather than agricultural, links suggesting that the first TuMV migrants to the Antipodes were in  
20 horticultural materials. In contrast, the isolates closest to the world-B2 and world-B3 migrants were from  
21 brassicas.

22 Our analysis of TuMV provides a significant snapshot of the evolution and emergence of a highly  
23 pathogenic virus in association with human immigration and agriculture history. It will also be important in the  
24 future to study the effect of large changes in TuMV host populations in these countries, such as those that may  
25 be caused by the recent widespread increase in area of crops of canola throughout Australia, and the  
26 emergence of this plant as one of the commonest roadside weeds in the regions where it is being grown. There  
27 is also a need to investigate TuMV in the brassica crops of Western Australia and the many native species of  
28 *Brassicaceae* in all of Oceania.

## 31 METHODS

32  
33 Virus isolates. The brassica crop-producing areas of Australia and New Zealand were surveyed during the  
34 growing seasons of 1994–2011. All collected samples were tested by direct double-antibody sandwich  
35 enzyme-linked immunosorbent assay (DAS-ELISA) using TuMV antiserum (Clark & Adams, 1977). Details of  
36 the isolates, their place of origin, original host plant, year of isolation, and host type are shown in Table S1,  
37 together with details of the isolates used in the analyses and for which complete genomic sequences have  
38 already been reported.

39  
40 It is essential to clone the viral isolates being studied before they are sequenced in studies of plant virus  
41 evolution because of the high frequency of mixed infections in the field, not only with other viruses but also  
42 with other isolates of the same virus (Ohshima *et al.*, 2002; Tomitaka & Ohshima, 2006). In the earlier studies,  
43 TuMV isolates were usually cloned by single lesion isolations. Consequently, we found very few mismatches in  
44 the sequences between the overlapping RT-PCR products (Nguyen *et al.*, 2013a). Moreover, cloning is required  
45 when attempting to analyse recombinational events and the genetic structure of populations. In present study,

1 all the isolates were inoculated to *C. quinoa* or *Nicotiana tabacum* cv. Samson and serially cloned through  
2 single lesions at least three times. They were propagated in *B. rapa* cv. Hakatasuwari or *N. benthamiana* plants.  
3 Plants infected systemically with each of the TuMV isolates were homogenized in 0.01 M potassium  
4 phosphate buffer (pH 7.0), and the isolates were mechanically inoculated to young plants of *Brassicaceae* plants  
5 (Nguyen *et al.*, 2013b). Inoculated plants were kept for at least four weeks in a glasshouse at 25°C.

6  
7 Viral RNA and sequencing. We determined the genomic sequences of TuMV collected in different areas of  
8 Australia and New Zealand. The viral RNAs were extracted from TuMV-infected *B. rapa* cv. Hakatasuwari or *N.*  
9 *benthamiana* leaves using Isogen (Nippon Gene, Japan). The RNAs were reverse transcribed by PrimeScript®  
10 *Moloney murine leukemia virus* (MMLV) reverse transcriptase (TakaraBio, Japan) and amplified using  
11 high-fidelity Platinum™ Pfx DNA polymerase (Invitrogen, USA). The products obtained by reverse transcription  
12 and polymerase chain reaction (RT-PCR) were separated by electrophoresis in agarose gels and purified using  
13 the QIAquick Gel Extraction Kit (Qiagen K. K, Japan). Sequences from each isolate were determined using four  
14 to five overlapping independent RT-PCR products to cover the complete genome. To ensure that they were from  
15 the same genome and were not from different components of a genome mixture, the sequences of the RT-PCR  
16 products of adjacent regions of the genome overlapped by around 200-350 nt. Each RT-PCR product was  
17 sequenced by primer walking in both directions using a BigDye Terminator v3.1 Cycle Sequencing Ready  
18 Reaction kit (Life Technologies, USA) and an Applied Biosystems 310 and 3130X Genetic Analyzer. Sequence  
19 data were assembled using BioEdit version 5.0.9 (Hall, 1999).

20  
21 Alignment of sequences. The genomic sequences of 229 isolates (Table S1) were used for phylogenetic and  
22 recombination analysis. Two sequences of Japanese yam mosaic virus (JYMV) (Fuji & Nakamae, 1999; 2000),  
23 one of scallion mosaic virus (ScaMV) (Chen *et al.*, 2002), one of narcissus yellow stripe virus (NYSV) (Chen *et al.*,  
24 2006) and two of narcissus late season yellows virus (NLSYV) (Lin *et al.*, 2012; Wylie *et al.*, 2014) were used as  
25 outgroup taxa because these are the other members of the TuMV phylogenetic group. The amino acid  
26 sequences of the polyproteins were aligned with the outgroup sequences using CLUSTAL\_X2 (Larkin *et al.*,  
27 2007) with TRANSALIGN (kindly supplied by Georg Weiller) to maintain the degapped alignment of the  
28 encoded amino acids. The aligned subsequences were then reassembled to form complete polyprotein  
29 sequences 8,922 nt long. The polyprotein sequences were then joined with aligned 5' and 3' NCR sequences of  
30 each isolate. This produced sequences 9,087 nt long, excluding the 35 nucleotides that were used to design the  
31 primer for RT-PCR amplification.

32  
33 Recombination analyses. Putative recombination breakpoints in all sequences were identified using RDP  
34 (Martin & Rybicki, 2000), GENECONV (Sawyer, 1999), BOOTSCAN (Salminen *et al.*, 1995), MAXCHI  
35 (Maynard-Smith, 1992), CHIMAERA (Posada & Crandall, 2001) and SISCAN programs (Gibbs *et al.*, 2000)  
36 implemented in the RDP4 package (Martin *et al.*, 2010) and also the original PHYLPRO (Weiller, 1998) and  
37 SISCAN version 2 (Gibbs *et al.*, 2000) programs. First, we checked for incongruent relationships using the  
38 programs implemented in RDP4. These analyses were done using default settings for the different detection  
39 programs and a Bonferroni-corrected *P*-value cut-off of 0.05 or 0.01. All isolates that had been identified as likely  
40 recombinants by the programs in RDP4, supported by three different methods with an associated *P*-value of  
41  $>1.0 \times 10^{-6}$ , were re-checked using the original PHYLPRO version 1 and SISCAN version 2. We checked 100- and  
42 50-nt slices of all sequences for evidence of recombination using these programs. These analyses also  
43 determined which non-recombinant sequences had regions that were closest to the regions of the recombinant  
44 sequences and hence indicated the likely lineages that provided those regions of the recombinant genomes. For  
45 convenience, we called these the 'parental isolates' of recombinants. Finally, TuMV sequences were also aligned

1 without outgroup sequences, producing sequences 9,710 nt long excluding the 35 nucleotides. We checked  
2 these for evidence of recombination using the programs described above.

3  
4 Phylogenetic analyses. The phylogenetic relationships of the aligned full and partial genomic sequences were  
5 inferred using the Neighbor-Net method in SPLITSTREE version 4.11.3 (Huson *et al.*, 2006) and using ML in  
6 PhyML version 3 (Guindon & Gascuel, 2003). For the ML analysis, we used the general time-reversible (GTR)  
7 model of nucleotide substitution, with rate variation among sites modelled using a gamma distribution and a  
8 proportion of invariable sites (GTR+ I+ $\Gamma_4$ ). This model was selected in R (Schliep, 2011) using the Bayesian  
9 information criterion, which has been shown to perform well in a variety of scenarios (Luo *et al.*, 2010). Branch  
10 support was evaluated by bootstrap analysis based on 1000 pseudoreplicates. The inferred trees were displayed  
11 by TREEVIEW (Page, 1996) and FigTree version 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Nucleotide  
12 and amino acid similarities were estimated using the Kimura two-parameter method (Kimura, 1980) and the  
13 Dayhoff PAM 001 matrix (Schwarz & Dayhoff, 1979), and the within-population diversities were assessed using  
14 MEGA version 6 (Tamura *et al.*, 2013).

15  
16 Estimation of substitution rates and divergence times. Bayesian phylogenetic analyses were performed in  
17 BEAST version 1.8.0 (Drummond *et al.*, 2012) to estimate the evolutionary rate and timescale of TuMV  
18 populations. Analyses were based on partial protein-encoding regions of HC-Pro (nt 1460-2494,  
19 corresponding to the positions in original UK 1 genome), P3 (nt 2591-3463) and N1b (nt 7208-8068).  
20 Recombinant sequences and some nucleotides from recombination ends were discarded from the three  
21 regions (see Fig. 2). We call these regions HC-Pro\*, P3\* and N1b\*, respectively. The sampling times of the  
22 sequences were used to calibrate the molecular clock. BFs were used to select the best-fitting molecular-clock  
23 model and coalescent priors for the tree topology and node times. Strict and relaxed (uncorrelated exponential  
24 and uncorrelated lognormal) molecular clocks (Drummond *et al.*, 2006) were compared, and five  
25 demographic models (constant population size, expansion growth, exponential growth, logistic growth, and  
26 the Bayesian skyline plot) were also compared.

27  
28 Posterior distributions of parameters, including the tree, were estimated by Markov Chain Monte Carlo  
29 (MCMC) sampling. Samples were drawn every  $10^4$  MCMC steps over a total of  $10^8$  steps, with the first 10% of  
30 samples discarded as burn-in. Acceptable sampling from the posterior and convergence to the stationary  
31 distribution were checked using the diagnostic software Tracer version 1.6  
32 (<http://tree.bio.ed.ac.uk/software/tracer/>). Tree files were generated with software included in the BEAST  
33 package and Bayesian maximum-clade-credibility (MCC) trees were displayed by FigTree version 1.4.2.

34  
35 Sampling times need to have a sufficient spread in relation to the substitution rate to allow reliable estimation  
36 substitution rates and divergence times from heterochronous sequence data (Drummond *et al.*, 2003). The  
37 temporal signal in our data sets was checked by comparing our rate estimates with those from ten  
38 date-randomized replicates. A data set was considered to have sufficient temporal structure when the mean  
39 rate estimate from the original data set was not contained in any of the 95% credibility intervals of the rates  
40 estimated from the date-randomized replicates. This follows the approach taken in previous studies of viruses  
41 (Duchêne *et al.*, 2014; Ramsden *et al.*, 2009).

42  
43 The spatial population dynamics of TuMV through time were inferred in BEAST using a diffusion model with  
44 discrete location states (Lemey *et al.*, 2009). This approach uses a model that describes the spatial migration of  
45 TuMV lineages throughout their demographic history. The most important pairwise diffusions can be

1 identified using BFs (Suchard *et al.*, 2001). We produced a graphical animation of the estimated  
2 spatio-temporal movements of TuMV lineages using SPREAD version 1.0.6 (Bielejec *et al.*, 2011) and Google  
3 Earth (<http://www.google.com/earth>).

4  
5 Demographic analyses. Haplotype and nucleotide diversities were estimated using DnaSP version 5.0 (Librado  
6 & Rozas, 2009). Haplotype diversity refers to the frequency and number of haplotypes (i.e. unique  
7 combinations of nucleotide polymorphisms) in the population. Nucleotide diversity estimates the average  
8 pairwise differences among sequences. Non-synonymous (dN) and synonymous (dS) substitution (dN/dS)  
9 ratios were calculated for each protein-encoding region using the Pamilo-Bianchi-Li method in MEGA version  
10 6 (Tamura *et al.*, 2013).

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21

1 REFERENCES

2

3 Bielejec, F, Rambaut, A, Suchard, M. A. & Lemey, P. (2011). SPREAD: spatial phylogenetic reconstruction of  
4 evolutionary dynamics. *Bioinformatics* 27, 2910–2912.

5 Chamberlain, E. E. (1936). Turnip-mosaic. A virus disease of crucifers. *New Zeal Jour Agr* 53, 321–330.

6 Chen, J., Zheng, H. Y., Chen, J. P. & Adams, M. J. (2002). Characterisation of a potyvirus and a potexvirus from  
7 Chinese scallion. *Arch Virol* 147, 683–693.

8 Chen, J., Lu, Y.-W., Shi, Y.-H., Adams, M. J. & Chen J.-P. (2006). Complete nucleotide sequence of the genomic RNA of  
9 Narcissus yellow stripe virus from Chinese narcissus in Zhangzhou city, China. *Arch Virol* 151, 1673–1677.

10 Chung, B. Y.-W., Miller, W. A., Atkins, J. F. & Firth, A. E. (2008). An overlapping essential gene in the *Potyviridae*.  
11 *Proc Natl Acad Sci USA* 105, 5897–5902.

12 Clark, M. F. & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent  
13 assay for the detection of plant viruses. *J Gen Virol* 34, 475–483.

14 Drummond, A. J., Pybus, O. G., Rambaut, A., Forsberg, R. & Rodrigo, A. G. (2003). Measurably evolving populations.  
15 *Trends Ecol Evol* 18, 481–488.

16 Drummond, A. J., Ho, S. Y. W., Phillips, M. J. & Rambaut, A. (2006). Relaxed phylogenetics and dating with  
17 confidence. *PLoS Biol* 4, e88.

18 Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the  
19 BEAST 1.7. *Mol Biol Evol* 29, 1969–1973.

20 Duchêne, S., Holmes, E. C. & Ho, S. Y. W. (2014). Analyses of evolutionary dynamics in viruses are hindered by a  
21 time-dependent bias in rate estimates. *Proc Roy Soc B* 281, 20140732.

22 Fletcher, J. D., Lister, R. A., Bulman, S. R. & Heenan, P. B. (2010). First record of *Turnip mosaic virus* in *Pachycladon*  
23 spp. (Brassicaceae): an endangered native plant species in New Zealand. *Australas Plant Dis Notes* 5, 9–10.

24 Fuji, S. & Nakamae, H. (1999). Complete nucleotide sequence of the genomic RNA of a Japanese yam mosaic  
25 virus, a new potyvirus in Japan. *Arch Virol* 144, 231–240.

26 Fuji, S. & Nakamae, H. (2000). Complete nucleotide sequence of the genomic RNA of a mild strain of Japanese  
27 yam mosaic potyvirus. *Arch Virol* 145, 635–640.

28 García-Arenal, F., Fraile, A. & Malpica, J. M. (2001). Variability and genetic structure of plant virus populations. *Ann*  
29 *Rev Phytopathol* 39, 157–186.

30 Gibbs, A. J., Fargette, D., Garcia-Arenal, F. & Gibbs, M. J. (2010). Time – the emerging dimension of plant virus  
31 studies. *J Gen Virol* 91, 13–22.

32 Gibbs, A. J., Mackenzie, A. M. Wei, K.-J. & Gibbs, M. J. (2006). The potyviruses of Australia. *Arch Virol* 153,  
33 1411–1420.

34 Gibbs, A. J., Ohshima, K., Gibbs, M. & García-Arenal, F. (2008). More about plant virus evolution; past, present  
35 and future. In *Origin and Evolution of Viruses*, 2nd ed, pp. 229–249. Edited by E. Domingo, C. R. Parrish & J. J.  
36 Holland. San Diego: Elsevier Academic Press.

37 Gibbs, A. J. & Ohshima, K. (2010). Potyviruses and the digital revolution. *Annu Rev Phytopathol* 48, 205–223.

38 Gibbs, M. J., Armstrong, J. S. & Gibbs, A. J. (2000). Sister-scanning: a Monte Carlo procedure for assessing signals  
39 in recombinant sequences. *Bioinformatics* 16, 573–582.

40 Guindon, S. & Gascuel, O. (2003). A simple fast and accurate algorithm to estimate large phylogenies by  
41 maximum likelihood. *Syst Biol* 52, 696–704.

42 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for  
43 Windows 95/98/NT. *Nucleic Acids Symp Ser* 41, 95–98.

44 Huson, D. H. & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 23,  
45 254–267.

1 Jenner, C. E., Sanchez, F., Nettleship, S. B., Foster, G. D., Ponz, F. & Walsh, J. A. (2000). The cylindrical inclusion gene of  
2 *Turnip mosaic virus* encodes a pathogenic determinant to the brassica resistance gene *TuRB01*. *Mol Plant Microbe*  
3 *Interact* 13, 1102–1108.

4 Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through  
5 comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.

6 King, A. M. Q., Adams, M. J., Carstens, E. B. & Lefkowitz, E. J. (2012). Virus Taxonomy: Ninth report of the  
7 international committee on taxonomy of viruses. In *Family Potyviridae*, pp. 1069–1077. San Diego: Elsevier  
8 Academic Press.

9 Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace,  
10 I.M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL X version 2.0. *Bioinformatics* 23, 2947–2948.

11 Lecoq, H., Wipf-Scheibel, C., Chandeysson, C., Lê Van, A., Fabre, F. & Desbiez, C. (2009). Molecular epidemiology of *Zucchini*  
12 *yellow mosaic virus* in France: an historical overview. *Virus Res* 141, 190–200.

13 Lefeuvre, P., Martin, D. P., Harkins, G., Lemey, P., Gray, A. J. A., Meredith, S., Lakay, F., Monjane, A., Lett, J.-M. & other  
14 authors (2010). The spread of tomato yellow leaf curl virus from the middle east to the world *PLoS Pathog* 6,  
15 e1001164.

16 Lemey, P., Rambaut, A., Drummond, A. J. & Suchard, M. A. (2009). Bayesian phylogeography finds its roots. *PLoS*  
17 *Comput Biol* 5, e1000520.

18 Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data.  
19 *Bioinformatics* 25, 1451–1452.

20 Lin, S.-Q., Shen, J.-G., Gao, F.-L., Cai, W., Huang, Z., Xie, L.-Y. & Wu, Z.-J. (2012). Complete genome sequence of  
21 narcissus latent season yellows virus infecting Chinese narcissus in China. *Arch Virol* 157, 1821–1824.

22 Luo, A., Qiao, H., Zhang, Y., Shi, W., Ho, S. Y. W., Xu, W., Zhang, A. & Zhu, C. (2010). Performance of criteria for  
23 selecting evolutionary models in phylogenetics: a comprehensive study based on simulated datasets. *BMC Evol*  
24 *Biol* 10, 242.

25 Martin, D. & Rybicki, E. (2000). RDP: detection of recombination amongst aligned sequences. *Bioinformatics* 16,  
26 562–563.

27 Martin, D. P., Lemey, P., Lott, M., Moulton, V., Posada, D. & Lefeuvre, P. (2010). RDP3: a flexible and fast computer  
28 program for analyzing recombination. *Bioinformatics* 26, 2462–2463.

29 Maynard-Smith, J. (1992). Analyzing the mosaic structure of genes. *J Mol Evol* 34, 126–129.

30 Nguyen, H. D., Tran, H. T. N. & Ohshima, K. (2013a). Genetic variation of the *Turnip mosaic virus* population of  
31 Vietnam: A case study of founder, regional and local influences. *Virus Res* 171, 138–149.

32 Nguyen, H. D., Tomitaka, Y., Ho, S. Y. W., Duchêne, S., Vetten, H.-J., Lesemann, D., Walsh, J. A., Gibbs, A. J. & Ohshima,  
33 K. (2013b). Turnip mosaic potyvirus probably first spread to Eurasian brassica crops from wild orchids about  
34 1000 years ago. *PLoS ONE* 8, e55336.

35 Ochoa Corona, F. M., Lebas, B. S. M., Elliott, D. R., Tang, J. Z. & Alexander, B. J. R. (2007). New host records and new  
36 host family range for *Turnip mosaic virus* in New Zealand. *Australas Plant Dis Notes* 2, 127–130.

37 Ogawa, T., Tomitaka, Y., Nakagawa, A. & Ohshima, K. (2008). Genetic structure of a population of *Potato virus Y*  
38 inducing potato tuber necrotic ringspot disease in Japan; comparison with North America and European  
39 populations. *Virus Res* 131, 199–212.

40 Ohshima, K., Yamaguchi, Y., Hirota, R., Hamamoto, T., Tomimura, K., Tan, Z., Sano, T., Azuhata, F., Walsh, J. A.,  
41 Fletcher, J., Chen, J., Gera, A. & Gibbs, A. J. (2002). Molecular evolution of *Turnip mosaic virus*: evidence of host  
42 adaptation, genetic recombination and geographical spread. *J Gen Virol* 83, 1511–1521.

43 Ohshima, K., Tomitaka, Y., Wood, J. T., Minematsu, Y., Kajiyama, H., Tomimura, K. & Gibbs, A. J. (2007). Patterns  
44 of recombination in turnip mosaic virus genomic sequences indicate hotspots of recombination. *J Gen Virol* 88,  
45 298–315.

- 1 Ohshima, K., Akaishi, S., Kajiyama, H., Koga, R. & Gibbs, A. J. (2010). Evolutionary trajectory of turnip mosaic  
2 virus populations adapting to a new host. *J Gen Virol* 91, 788–801.
- 3 Page, R. D. M. (1996). Treeview: an application to display phylogenetic trees on personal computers. *Comput*  
4 *Appl Biosci* 12, 357–358.
- 5 Posada, D. & Crandall, K. A. (2001). Evaluation of methods for detecting recombination from DNA sequences:  
6 computer simulations. *Proc Natl Acad Sci USA* 98, 13757–13762.
- 7 Ramsden, C., Holmes, E. C. & Charleston, M. A. (2009). Hantavirus evolution in relation to its rodent and  
8 insectivore hosts: no evidence for codivergence. *Mol Biol Evol* 26, 143–153.
- 9 Rocha, C. S., Castillo-Urquiza, G. P., Lima, A. T. M., Silva, F. N., Xavier, C. A. D., Hora-Júnior, B. T., Beserra-Júnior, J. E. A.,  
10 Malta, A. W. O., Martin, D. P. & other authors (2013). Brazilian begomovirus populations are highly recombinant,  
11 rapidly evolving, and segregated based on geographical location. *J Virol* 87, 5784–5799.
- 12 Salminen, M. O., Carr, J. K., Burke, D. S. & McCutchan, F. E. (1995). Identification of breakpoints in intergenotypic  
13 recombinants of HIV type 1 by Bootscanning. *AIDS Res Hum Retroviruses* 11, 1423–1425.
- 14 Samuel, G. (1931). Summary of plant disease records in south Australia for the two years ending June 30, 1930.  
15 *So Aust Dept Agr Jour* 34, 746.
- 16 Sawyer, S. A. (1999). GENECONV: a computer package for the statistical detection of gene conversion,  
17 Distributed by the Author. Department of Mathematics. Washington University, St Louis, available at  
18 <http://www.math.wustl.edu/~sawyer>.
- 19 Schliep, K. P. (2011). Phangorn: phylogenetic analysis in R. *Bioinformatics* 27, 592–593.
- 20 Schwarz, R. & Dayhoff, M. (1979). Matrices for detecting distant relationships. In *Atlas of protein sequences*, pp. 353–358.  
21 Edited by M. Dayhoff. Washington, DC: National Biomedical Research Foundation.
- 22 Schwinghamer, M. W., Schilg, M. A., Walsh, J. A., Bambach, R. W., Cossu, R. M., Bambridge, J. M., Hind-Lanoiselet, T.  
23 L., McCorkell, B. E. & Cross, P. (2014). *Turnip mosaic virus*: potential for crop losses in the grain belt of New South  
24 Wales, Australia. *Australas Plant Pathol* Published online 2014 July 2 DOI: 10.1007/s13313-014-0304-9.
- 25 Seo, J. K., Ohshima, K., Lee, H. G., So, M., Choi, H. S., Lee, S. H., Sohn, S. H. & Kim, K. H. (2009). Molecular variability  
26 and genetic structure of the population of *Soybean mosaic virus* based on the analysis of complete genome  
27 sequences. *Virology* 393, 91–103.
- 28 Suchard, M. A., Weiss, R. E. & Sinsheimer, J. S. (2001). Bayesian selection of continuous- time Markov chain  
29 evolutionary models. *Mol Biol Evol* 18, 1001–1013.
- 30 Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics  
31 analysis version 6.0. *Mol Biol Evol* 30, 2725–2729.
- 32 Tomimura, K., Gibbs, A. J., Jenner, C. E., Walsh, J. A. & Ohshima, K. (2003). The phylogeny of *Turnip mosaic virus*;  
33 comparisons of 38 genomic sequences reveal a Eurasian origin and a recent 'emergence' in east Asia. *Mol Ecol*  
34 12, 2099–2111.
- 35 Tomimura, K., Späk, J., Katis, N., Jenner, C. E., Walsh, J. A., Gibbs, A. J. & Ohshima, K. (2004). Comparisons of the  
36 genetic structure of populations of *Turnip mosaic virus* in West and East Eurasia. *Virology* 330, 408–423.
- 37 Tomitaka, Y. & Ohshima, K. (2006). A phylogeographic study of the *Turnip mosaic virus* population in East Asia  
38 reveals an 'emergent' lineage in Japan. *Mol Ecol* 15, 4437–4457.
- 39 Tomitaka, Y., Yamashita, T. & Ohshima, K. (2007). The genetic structure of populations of *Turnip mosaic virus* in  
40 Kyushu and central Honshu, Japan. *J Gen Plant Pathol* 73, 197–208.
- 41 Visser, J. C., Bellstedt, D. U. & Pirie, M. D. (2012). The recent recombinant evolution of a major crop pathogen,  
42 *Potato virus Y*. *PLoS ONE* 7, e50631.
- 43 Wace, N. (1985). The isolated continent. In *Pests and parasites as migrants*, pp. 3–22. Edited by A. Gibbs & R.  
44 Meischke. Cambridge: Cambridge University Press.
- 45 Walsh, J. A. & Jenner, C. E. (2002). *Turnip mosaic virus* and the quest for durable resistance. *Mol Plant Pathol* 3,



1 289–300.  
2 Weiller, G. F. (1998). Phylogenetic profiles: a graphical method for detecting genetic recombinations in  
3 homologous sequences. *Mol Biol Evol* 15, 326–355.  
4 Wertheim, J. O. & Kosakovsky Pond, S. L. (2011). Purifying selection can obscure the ancient age of viral lineages.  
5 *Mol Biol Evol* 28, 3355–3365.  
6 Wylie, S. J., Li, H., Sivasithamparam, K. & Jones, M. G. K. (2014). Complete genome analysis of three isolates of  
7 narcissus late season yellows virus and two of narcissus yellow stripe virus: three species or one? *Arch Virol* 159,  
8 1521–1525.  
9 Yasaka, R., Nguyen, H. D., Ho, S. Y. W., Duchêne, S., Korkmaz, S., Katis, N., Takahashi, H., Gibbs, A. J. & Ohshima, K.  
10 (2014). The temporal evolution and global spread of *Cauliflower mosaic virus*, a plant pararetrovirus. *PLoS ONE*  
11 9, e85641.  
12 Zubareva, I. A., Vinogradova, S. V., Gribova, T. N., Monakhos, S. G., Skryabin, K. G. & Ignatov, A. N. (2013). Genetic  
13 diversity of *Turnip mosaic virus* and the mechanism of its transmission by Brassica seeds. *Doklady Biochem.*  
14 *Biophys* 450, 119–122.  
15

1 Figure legends

2

3 Fig. 1. Maps showing the provenance of the turnip mosaic virus isolates from Australia and New Zealand that  
4 were studied. Dots on the map correspond to the isolates listed in Table S1.

5

6 Fig. 2. Recombination maps of turnip mosaic virus genomes of Australian and New Zealand isolates. The  
7 estimated nucleotide positions of the recombination sites and those in parentheses are shown relative to the 5'  
8 end of the genome using the numbering of the aligned sequences used in the present study and the UK 1 isolate  
9 (Jenner *et al.*, 2000), respectively. Vertical arrows and lines show estimated recombination sites (listed in Table  
10 S2). The grey and dot boxes indicate basal-B and world-B parents, respectively. The horizontal arrows show  
11 the regions (A, B and C) used to infer trees from non- and intralineage recombinant sequences (shown in  
12 Supplementary Fig. S2). The recombination sites newly identified in the present study (black) or those  
13 identified in earlier studies (red) are separately listed.

14

15 Fig. 3. Bayesian maximum-clade-credibility chronogram inferred from the protein-encoding region of turnip  
16 mosaic virus. The tree was calculated from the 186 non-recombinant isolates of partial protein 3 (P3\*) (nt  
17 2591-3463, corresponding to the positions in original UK 1 genome) sequences. The non-Australian and  
18 non-New Zealand (sub)groups of basal-B1, basal-BR, Asian-BR and world-B1 are collapsed. Horizontal blue  
19 bars represent the 95% highest posterior density interval (HPD) intervals of estimates of node ages. The bar  
20 graph shows the root state posterior probabilities for each location (colored bars). Gray bars show probabilities  
21 obtained with 10 randomizations of the tip locations.

22

23 Fig. 4. Plausible historical migration pathways of turnip mosaic virus inferred using partial helper-component  
24 proteinase (HC-Pro\*), protein 3 (P3\*) and nuclear inclusion b (NIB\*) regions. Details of the regions are given in  
25 the Methods. Migration routes only for Australia and New Zealand are shown, and only when supported by a  
26 Bayes factor (BF) greater than 10. Only the migration pathways for basal-B2 (a), world-B2 (b) and world-wB3  
27 (c) isolates are shown. For each subgroup migration, the BF and estimated age in years before present (YBP)  
28 are shown (d). The 95% highest posterior density interval (HPD) interval for each age estimate is given in  
29 parentheses. We analysed basal-B group isolates instead of basal-B2 subgroup isolates because too few  
30 isolates belong to basal-B2 subgroup, and only the BFs from the basal-B2 subgroup are listed. BF values can be  
31 interpreted as follows:  $10 \leq \text{BF} < 30$ , strong support;  $30 \leq \text{BF} < 100$ , very strong support; and  $\text{BF} \geq 100$ , decisive  
32 support.

Table 1. Details of the data sets used for estimation of nucleotide substitution rate and time to the most recent common ancestor for turnip mosaic virus

Parameter	Protein encoding region*		
	Helper-component proteinase	Protein 3	Nuclear inclusion b
Best-fit substitution model	GTR + I + $\Gamma_4$	GTR + I + $\Gamma_4$	GTR + I + $\Gamma_4$
Best-fit molecular clock model	Relaxed uncorrelated exponential	Relaxed uncorrelated exponential	Relaxed uncorrelated exponential
Best-fit population growth model	Constant size	Constant size	Constant Size
Sequence length (nt)	927	897	891
No. of sequences	180	186	182
Sampling date range	1968-2012	1970-2012	1968-2012
Chain length (in millions)	100	100	100
TMRCAt (years)			
All isolates	610 (233-1156)‡	806 (274-1630)	679 (205-1502)
Australia			
basal-B2 subgroup	80 (119-52) [n=12]§	44 (65-25) [n=13]	36 (51-19) [n=2]
world-B2 subgroup	14 (20-9) [n=2]	9 (29-5) [n=2]	NA   [n=0]
world-B3 subgroup	27 (36-16) [n=2]	NA [n=1]	46 (67-28) [n=14]
New Zealand			
basal-B2 subgroup	14 (29-4) [n=3]	16 (28-4) [n=3]	22 (31-12) [n=3]
world-B2 subgroup	56 (83-30) [n=13]	16 (23-9) [n=10]	17 (24-11) [n=9]
world-B3 subgroup	NA [n=1]	NA [n=1]	68 (100-36) [n=5]
Substitution rate (nt/site/year)	$1.47 \times 10^{-3}$ ( $1.08 \times 10^{-3}$ - $1.89 \times 10^{-3}$ )	$1.35 \times 10^{-3}$ ( $9.50 \times 10^{-4}$ - $1.77 \times 10^{-3}$ )	$1.30 \times 10^{-3}$ ( $9.07 \times 10^{-4}$ - $1.77 \times 10^{-3}$ )
dN/dS¶	0.025	0.120	0.030
No. of variable sites#	511	536	493

\*Partial helper-component (HC-Pro\*), Protein 3 (P3\*) and nuclear inclusion b (Nib\*) regions (see Methods).

†Time to the most recent common ancestor (years).

‡95% highest posterior density interval in parentheses.

§The number of isolates in square brackets.

||Not available.

¶Non-synonymous (dN) and synonymous (dS) substitution (dN/dS) ratios were calculated for three protein-encoding regions using the Pamilo-Bianchi-Li method.

#The number of variable sites.

Table 2. The estimate of the time of recombination events of turnip mosaic virus in Australia and New Zealand

Recombination site*	Parent (5' × 3')	Recombinant type†	Recombination age (YBP)‡	Stem age (YBP)	Crown age (YBP)
Australia					
nt 818	world-B3 × basal-B2	Inter	50-10	50-18	23-10
nt 1080	world-B2 × basal-B2	Inter	54-13	54-21	50-13
nt 1341	world-B3 × basal-B2	Inter	48-21	48-25	40-21
nt 1851	basal-B2 × basal-B2	Intra	35-9	35-14	22-9
nt 2530	basal-B2 × basal-B2	Intra	48-23	48-25	37-23
nt 2742	world-B3 × basal-B2	Inter	38-14	38-14	N/A§
nt 3475	basal-B2 × world-B3	Inter	51-22	51-26	49-22
New Zealand					
nt 5602	world-B3 × world-B3	Intra	49-20	49-37	46-20
nt 5665	world-B2 × world-B3	Intra	22-11	22-11	N/A
nt 6019	basal-B2 × basal-B2	Intra	75-19	75-28	70-19
nt 8071	world-B2 × world-B3	Intra	20-10	20-14	19-10
Both countries					
nt 1174	world-B2 × world-B2	Intra	27-14	27-18	24-14
nt 5219	world-B2 × world-B2	Intra	24-13	24-17	21-13
nt 6132	world-B2 × world-B3	Intra	28-16	28-20	25-16

\*The ages of major recombination sites in Australia and New Zealand were estimated with reference to the results of Bayesian phylogenetic analyses shown in Figs. S6 (a) and S6 (b), respectively. The common recombination sites in both countries were estimated from the tree including all isolates (data not shown). Nucleotide positions show locations of individual genes numbered as in the original UK 1 genome (Jenner *et al.*, 2000).

†Inter; interlineage recombination site, Intra; intralineaage recombination site.

‡The oldest and youngest ages are shown. The oldest and the youngest ages were estimated from the stem and crown ages, respectively. Estimates are given in years before present (YBP).

§Not available