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DOI
10.1111/1462-2920.13281

Publication date
2016

Document Version
Final published version

Published in
Environmental Microbiology

License
Article 25fa Dutch Copyright Act

Link to publication

Citation for published version (APA):
Vlaardingerbroek, I., Beerens, B., Rose, L., Fokkens, L., Cornelissen, B. J. C., & Rep, M. (2016). Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in Fusarium oxysporum. *Environmental Microbiology*, *18*(11), 3702-3713. https://doi.org/10.1111/1462-2920.13281

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Download date:16 Aug 2022

environmental microbiology

Environmental Microbiology (2016) **18**(11), 3702–3713 doi:10.1111/1462-2920.13281

Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in *Fusarium oxysporum*

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Summary

Horizontal transfer of supernumerary or lineage-specific (LS) chromosomes has been described in a number of plant pathogenic filamentous fungi. So far it was not known whether transfer is restricted to chromosomes of certain size or properties, or whether 'core' chromosomes can also undergo horizontal transfer. We combined a directed and a non-biased approach to determine whether such restrictions exist. Selection genes were integrated into the genome of a strain of *Fusarium oxysporum* pathogenic on tomato, either targeted to specific chromosomes by homologous recombination or integrated randomly into the genome. By testing these strains for transfer of the marker to another strain we could confirm transfer of a previously described mobile pathogenicity chromosome. Surprisingly, we also identified strains in which (parts of) core chromosomes were transferred. Whole genome sequencing revealed that this was accompanied by the loss of the homologous region from the recipient strain. Remarkably, transfer of the mobile pathogenicity chromosome always accompanied this exchange of core chromosomes.

Introduction

In many filamentous fungi a distinction can be made between 'core' chromosomes, which are present in all strains within the species, and chromosomes composed primarily of DNA sequences not found in all strains of the species. These extra chromosomes are known as conditionally dispensable, accessory, supernumerary, mini- or B chromosomes (Covert, 1998). In *Fusarium oxysporum* they are known as lineage-specific (LS) chromosomes (Ma *et al*., 2010). In both *F. oxysporum* and other pathogenic fungi some LS chromosomes carry genes associated with pathogenicity (Croll and McDonald, 2012; Raffaele and Kamoun, 2012).

Comparison of genomes of the tomato pathogen *F. oxysporum* f. sp. *lycopersici* 4287 (*Fol*4287*)* and *Fusarium verticillioides* revealed that large sub-telomeric regions of chromosome 1 and 2 and all of chromosomes 3, 6, 14 and 15 of *Fol4287* do not have syntenic chromosomes or regions in *F. verticillioides* and are, therefore, designated as LS regions (Ma *et al*., 2010). They are distinguished from the core genome by a much higher density of transposable elements (TEs) and lower gene density, with few or no housekeeping genes. Most genes for small proteins secreted in xylem of infected plants (*SIX* genes) (Houterman *et al*., 2007), are located on a single ~2-Mb LS chromosome, chromosome 14 in *Fol*4287, which is present in all isolates that cause tomato wilt. Upon co-incubation of *F. oxysporum* f.sp. *lycopersici* strain *Fol*007 with the non-pathogenic strain *Fo*47, transfer of this LS chromosome, as well as the smallest LS chromosome, from *Fol*007 to *Fo*47 has been observed (Ma *et al*., 2010). In that study the chromosome corresponding to LS chromosome 14 of the reference strain *Fol*4287 was labelled with the zeocin resistance gene (*BLE*) whereas *Fo*47 contained a hygromycin resistance gene (*HPH*) inserted randomly into the genome, allowing selection of colonies carrying genetic material from both parental strains. The chromosome corresponding to chromosome 14 of *Fol*4287 is considered a 'pathogenicity chromosome' as it confers pathogenicity to tomato when transferred to *Fo*47. Nine double resistant strains were analysed, each showing the same karyotype as *Fo*47 with the addition of the pathogenicity chromosome; two of these strains also contained a second, smaller LS chromosome of *Fol*007. Transfer of *Fo*47 material to *Fol*007 was not observed (Ma *et al*., 2010).

Horizontal Chromosome Transfer (HCT) of LS chromosomes has been described in other species of pathogenic

Received 25 November, 2015; revised 22 February, 2016; accepted 22 February, 2016. *For correspondence. E-mail m.rep@uva.nl; Tel. 31205257764.

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filamentous fungi as well. In one of the earliest reports of HCT a 2-Mb chromosome was transferred between non-compatible strains of *Colletotrichum gloeosporioides* – although in this case transfer of pathogenicity was not observed (He *et al*., 1998). Transfer was found by testing for transfer of a marker located on the 2-Mb chromosome. Transfer of the marker was not observed when the marker was not targeted to the LS chromosome but randomly integrated in nine independent strains, suggesting that not all chromosomes are amenable for transfer in *Colletotrichum*.

HCT has also been suggested to have played a role in evolution of *Alternaria alternata* pathotypes, in which host-specific toxin genes reside on LS chromosomes (Akagi *et al*., 2009). When different pathotypes are grouped based on genes on the core genome the pathotypes do not group together, suggesting HCT might be involved in the distribution of LS chromosomes within the species. Protoplast fusions of two pathotypes yielded a hybrid containing LS chromosomes from both parents, supporting the possibility of horizontal transfer (Akagi *et al*., 2009).

Nectria haematococca (anamorph *Fusarium solani*) contains a cluster of pea pathogenicity (*PEP*) genes located on a LS chromosome (Han *et al*., 2001). This cluster is present not only in *N. haematococca* but also in a strain of *F. oxysporum* f. sp. *pisi*, a pathogen of pea, and in *Neocosmospora boniensis* which was not known as a pathogen on pea but demonstrated to be able to cause disease (Temporini and VanEtten, 2004). Based on these observations it was suggested that the distribution of the *PEP* genes is not the result of shared ancestry and loss in several lineages but the result of horizontal transfer of genetic material. Taken together, these observations suggest that horizontal transfer of pathogenicity genes and/or pathogenicity chromosomes between incompatible strains or even different species plays an important role in the evolution of pathogenicity in filamentous fungi.

Slow-growing heterokaryons consisting of two incompatible strains have been observed in *C. gloeosporioides* and are suggested to play a role in HCT (Manners and He, 2011). In these heterokaryons genomes from both strains co-exist in the same mycelium, potentially enabling transfer. Suppression of heterokaryon incompatibility following fusion of conidial anastomosis tubes, a specialized form of hyphal fusion (Ishikawa *et al*., 2012), has been suggested to result in cytoplasmic continuity, creating a pathway for HCT (Mehrabi *et al*., 2011). Conidial anastomosis tube fusion could lead to the slow-growing heterokaryons observed by Manners and He (2011).

While heterokaryosis is likely required for HCT, little is known about the cellular and molecular processes underlying HCT or possible requirements such as chromosome size and structure. Most filamentous fungi have closed mitosis where the nuclear envelope is at least partially maintained during cell division, although equilibration between nucleoplasm and cytoplasm does occur (De Souza and Osmani, 2007). Traversing the nuclear envelope could favour smaller elements (He *et al*., 1998) potentially excluding larger chromosomes from transfer, although it is hard to imagine how even a small chromosome (with nucleosomes) could travel through a nuclear pore. Alternatively, nuclear fusion followed by selective degradation of all but the transferred chromosome could result in chromosome transfer (He *et al*., 1998).

Structural differences have been noted between core and LS chromosomes that could affect their propensity for horizontal transfer. The high TE content of LS chromosomes (Ma *et al*., 2010) or the enrichment of certain types of histone modifications in non-syntenic regions, as described in *Fusarium graminearum* (Connolly *et al*., 2013), are potential prerequisites for transfer. Apart from these structural properties transfer could be facilitated by a specific genetic element present on LS chromosomes (Rosewich and Kistler, 2000).

To work towards answering these questions, we set out to identify *Fol*4287 chromosomes that can be transferred to another strain. We used donor strains derived from *Fol*4287 with a selection marker either targeted to a specific chromosome or randomly integrated into the genome. Upon co-incubation with a recipient strain that was labelled with a second selection marker, progeny strains were selected for the stable presence of both markers. Analysis of the strains containing both markers not only confirmed the transfer of chromosome 14 but surprisingly revealed exchange of core genomic regions as well: a core chromosome (or a region thereof) of the recipient was replaced by the homologous region originating from the donor. Transfer of chromosome 14 always accompanied the exchange of core regions.

Results

A targeted approach confirms transfer of chromosome 14 from the tomato-infecting strain Fol4287

To find out whether transfer is restricted to small LS chromosomes, we first tested several chromosomes of the reference strain *Fol*4287 for transfer, using chromosome 14 as positive control. Chromosome 'donor' strains were created by targeting an *HPH-GFP* gene fusion to chromosomes 1, 12, 14 and 15 of *Fol*4287. *HPH* allows for selection of transfer of the marker from the donor (*Fol*4287) to the recipient (*Fo*47) while *GFP* allows for easy confirmation of transfer by microscopic examination. *Fol*4287 was transformed with one of the plasmids p1, p12, p14 or p15 to tag specific chromosomes. The markers were targeted to non-coding sequence not containing any repetitive sequences. Each plasmid was created by cloning two adjacent ~1-kb DNA fragments from the target chromosome into the multiple cloning site

Table 1. Position of markers on chromosomes.

Chromosome	Supercontig	Position	Size of supercontig
1	14	963040	1603705
12	23	278722	937052
14	22	396619	1030612
15	24	24947	957384

of pPK2-HPH-GFP (Michielse *et al*., 2009) such that recombination would result in the insertion of the marker between the two ~1-Kb fragments (see Table 1 for positions of insertion of the marker). For chromosome 1 and 14 two independent *in-locus* transformants were obtained, 1HG1 and 1HG2, and 14HG1 and 14HG2 respectively. For chromosome 12 and 15, one transformant each was obtained, called 12HG and 15HG respectively. Recipient strains were created by transforming *Fo*47 with pGRB (Vlaardingerbroek *et al*., 2015) containing *RFP* fused to *BLE*, the zeocin resistance gene. Two independent transformants were used, *Fo*47GRB1 and *Fo*47GRB2, each with the marker randomly inserted in the genome. Each donor strain was co-incubated with both recipients on rich Potato Dextrose Agar (PDA) as well as on synthetic minimal medium Czapex Dox Agar (CDA). For each potential donor 10 plates were used of each medium, split evenly between the two independent recipient strains.

Co-incubation of the two 14HG strains with the *Fo*47GRB strains on CDA medium yielded both small and large colonies on double selective medium. In total 78 large colonies were found on 9 plates. One large colony per plate was picked and monospored. Of these 9 colonies, 7 were confirmed to express both fluorescent markers and showed growth on both selective media confirming the presence of both markers. This suggests that the majority of the 78 large colonies are positive for both markers. Small colonies were present on all 20 plates resulting from the co-incubation of either 14HG1 or 14HG2 with the recipient strains on CDA. One colony was picked from each plate. Of these only two expressed both markers and grew on both media. From this we conclude that the majority of the, in total, 440 small colonies originating from co-incubation on CDA plates are false positives. However, if 10% of the colonies represent real transfer events there were still ~40 such events. From the plates on which spores had grown collected from PDA co-incubation plates, a plug was taken randomly as no individual colonies could be distinguished; the plates were overgrown with mycelium. Of the 20 plugs tested, 12 showed the presence of both markers and growth on both selective media. From this, we conclude that transfer of chromosome 14 to Fo47 recipient strains is a relatively common event and not restricted to a specific donor strain. Calculating the frequency of transfer was not possible in a reliable way, because there is no way to distinguish individual transfer events from an early event, leading to the production of many double resistant spores. What could be determined is that double resistant progeny were generated on almost all co-incubation plates.

Co-incubation of 15HG and the recipient strains followed by transfer to double selective medium resulted in the emergence of seemingly double resistant colonies. A total of 46 colonies were found after co-incubation on CDA while co-incubation on PDA resulted in overgrown double selective plates, similar to the situation with the marker on chromosome 14. All colonies originating from co-incubation on CDA as well as two plugs taken from each plate inoculated with spores from the PDA co-incubation plates, were further examined. None of the 46 colonies and 40 plugs tested showed resistance to both antibiotics nor the presence of both fluorescent markers. From this, we conclude that stable transfer of chromosome 15 of *Fol*4287 occurs either very rarely or not at all.

Similar results were obtained for chromosomes 1 and 12. Co-incubation of 12HG with *Fo*47GRB1 and *Fo*47GRB2 on CDA and PDA, followed by transfer to double selective medium did yield seemingly double resistant colonies. A total of 52 and 33 colonies were found, respectively. However, as was the case with the marker on chromosome 15, none of the 85 colonies tested was confirmed to be double resistant or double fluorescent. Upon co-incubation of the two 1HG strains with recipient strains, no double resistant progeny strains were found. So while chromosome 12 is similar in size to chromosome 14, transfer is much less likely to occur.

A non-targeted approach to identify chromosomes that can be transferred reveals exchange of core regions

Since the targeted approach did not lead to the identification of transferrable chromosomes other than chromosome 14, a large scale, non-biased approach was chosen to test transfer of other genomic regions. First, a collection was made of transformants with a selection marker integrated randomly in the genome and tested for transfer (see left side of Fig. 1 for schematic representation). *Fol*4287 was transformed with plasmid pRW1p (Houterman *et al*., 2008), which contains the *BLE* zeocin resistance gene. In total 269 individual transformants were obtained, a number high enough to expect insertions in all chromosomes, including the smaller chromosomes, assuming integration is not highly biased towards certain chromosomes. To assess their capability to act as donor of the marker gene, each individual transformant was co-incubated with strain *Fo*47H1 containing the *HPH* hygromycin resistance cassette randomly inserted into the genome. The latter strain has previously been shown to be capable of accepting chromosomes from *Fol*007

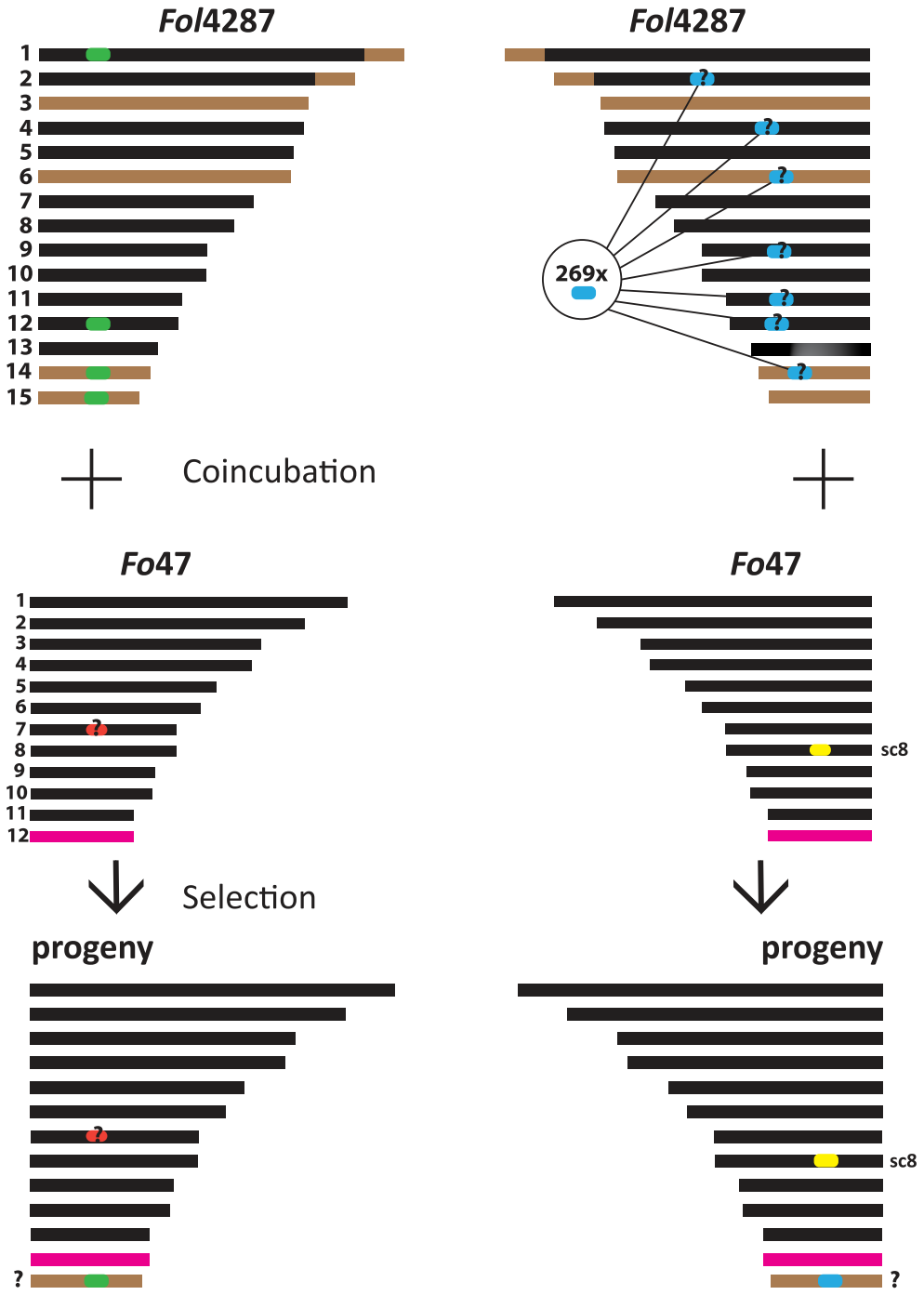

Fig. 1. Graphical summary of testing chromosomes for transfer and screening. Black bars indicate core chromosomes which are syntenic between strains, brown bars indicate *Fol*4287 LS chromosomes and the magenta bars represent the *Fo*47 LS chromosome. Most differences in karyotype between these two strains are the result of differences in LS content. The left side of the figure represents a targeted approach to test specific chromosomes for transfer. The right side gives an overview of a screen designed to identify chromosomes or regions amenable to transfer. Locations of the markers in the different strains are indicated with smaller bars. In the targeted approach (left side) chromosomes 1, 12, 14 and 15 were all targeted for insertion with a *HPH-GFP* fusion gene (green) and the resulting strains tested for transfer of the targeted chromosome to *Fo*47 with a random integration of *BLE-RFP* (red). Transfer capability of other *F. oxysporum* chromosomes was tested with a screen (right side). In the screen 269 individual transformants with the marker gene *BLE* (blue) integrated in a random location in the genome were tested for transfer of the marker to the non-pathogenic strain *Fo*47 carrying the *HPH* resistance gene (yellow). Double resistant progeny strains generated by co-cultivation of these strains were tested for the presence of chromosomes from both parents. Combining the results from these approaches it can be determined if transfer is restricted to LS chromosomes and if any size restrictions exist.

(Ma *et al*., 2010). After co-incubation, spores were collected and transferred to plates containing both drugs. These were then screened for the emergence of double resistant colonies, indicative for the transfer of the *BLE* marker from the donor to the recipient.

The initial screening yielded 14 transformants capable of producing double resistant colonies upon co-incubation with *Fo*47H1. All these were re-tested in a comprehensive co-incubation experiment as described above. To allow detection of rare marker transfer events 20 plates were used for each combination, divided equally over PDA and CDA. Six of the 14 transformants again yielded colonies growing on double selective medium after co-incubation. Upon monosporing, polymerase chain reaction (PCR) analysis confirmed the presence of both the *BLE* and the *HPH* marker genes in the progeny strains of three of these transformants, indicating their capacity to donate the *BLE* marker to recipient *Fo47*. The numbers of double resistant colonies found after co-cultivation differed for these three donor strains, designated donor A, B and C. For donors A and B only two individual colonies (designated a1 and a2 and b1 and b2 respectively) were found on the 20 plates used in this experiment. For donor C, on the other hand, transfer was a common occurrence. PDA plates were overgrown with colonies to the extent that discrimination between individual colonies was no longer possible. The number of double resistant colonies growing after co-incubation on CDA was in the range of dozens of colonies per plate. Transfer of the *BLE* marker from donor C was confirmed for 10 individual colonies. Repetition of the experiment yielded two additional colonies for donor A containing both markers genes (a3 and a4); for donor B no additional colonies were obtained. The result for donor C was the same as before, with an abundance of double resistant colonies. Three of these colonies were picked for further analysis (c1–c3).

Progeny strains from all three donor strains contain chromosome 14-specific markers

To assess the presence of genomic regions other than the marker sequences, the genomes of all progeny strains originating from each of the three *BLE* donors were first tested for the presence of *SIX1* and *SIX6*, which reside on different parts of chromosome 14. All strains were found to be positive for both *SIX* genes. In an earlier study in which the homolog of chromosome 14 from strain *Fol007* was shown to be amenable for transfer (Ma *et al*., 2010), a smaller LS chromosome co-transferred in two out of nine cases. This prompted us to look for other LS sequences, besides *SIX1* and *SIX6*, in the progeny strains. We used sets of primers targeting specific insertions of Foxy transposons (Ma *et al*., 2010) in the LS regions of the *Fol*4287 genome (Fig. 1). For the LS regions of chromosome 1 and 2 one marker was used for each and at least two markers were used for chromosomes 3, 6, 14 and 15. All strains tested contained the two additional markers for chromosome 14; presence of markers for other LS regions was not observed (Supporting Information Fig. S1). This suggests that no other LS sequences were transferred. To further substantiate the presence of chromosome 14 sequences, four additional genes located on chromosome 14, *SIX2, SIX3, SIX7* and *ORX1* (Ma *et al*., 2010), were tested and found to be present. In summary, in all progeny strains markers were present covering the full length of chromosome 14 suggesting transfer of this chromosome to the recipient strain in all cases (Supporting Information Fig. S2).

CHEF gel analysis confirms transfer of chromosome 14

To confirm transfer of chromosome 14 from the pathogenic donor to the non-pathogenic recipient and to observe other

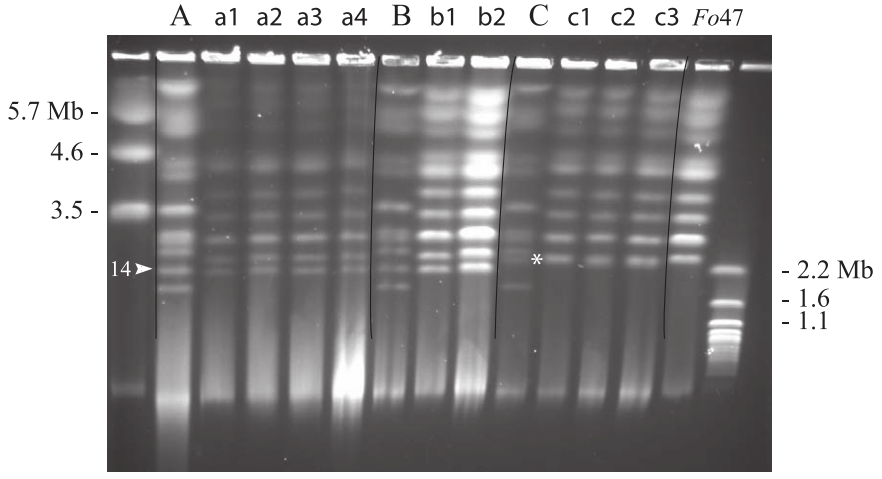

Fig. 2. Karyotyping of donor and recipient strains confirms transfer. CHEF gel analysis of donor and recipient strains reveals the presence of chromosome 14 (white arrow) from donor A in recipients a1–a4 and from donor B in b1 and b2. In recipients c1–c3 no extra chromosome is visible compared with *Fo*47H1, however, chromosome 14 in donor C is larger than for the other donor strains (left of the asterisk) - this results in this chromosome co-migrating with the smallest chromosome from *Fo*47. This results in the double band seen in these progeny strains (right of the asterisk), and is just as intense as the band above (also consisting of two chromosomes co-migrating). Several marker genes on chromosome 14 were shown to be present in these strains indicating transfer of the entire chromosome 14.

possible changes in karyotype, CHEF gel analysis was performed. Progeny strains from donors A and B had the same karyotype as the non-pathogenic recipient with the addition of a smaller chromosome of similar size as chromosome 14 from their respective donors (Fig. 2). The karyotype of donor C slightly differs from those of donors A and B: chromosome 14 in donor C seems to be larger than in the other two, running at the same position as the smallest chromosome of the recipient (Fig. 2 asterisk). No separate band could be seen for chromosome 14 in progeny strains c1–c3, however, the presence of the *SIX* genes suggests that chromosome 14 is present in the progeny strains. We suggest that chromosome 14 is present in the same position as the smallest chromosome of *Fo*47 in the CHEF gel. This is consistent with the increase in intensity of this band in progeny strains c1–c3 compared with the other progeny strains and their respective positions in the CHEF gel. In a1–a4 and b1–b2 the band for the smallest chromosome is less intense than the band above it. In c1–c3 intensity is the same for the two bands corresponding to the smallest chromosomes (Fig. 2).

Whole genome sequencing reveals exchange of core genomic regions

To determine more precisely the insertion site of the markers in the genome of the three donor strains and the recipient strain, and to identify any sequences originating from the donor other than chromosome 14, the genomes of progeny strains a1, b1 and c1 and donor C were sequenced with an average coverage of at least 50X. To determine the site of insertion of the markers, the sequence of the T-DNA from pRW1p, the construct present in the donor strains, was added to the *Fol*4287 genome sequence as a separate contig. Then the reads from each of the progeny strain genomes were mapped to this assembly. Pair mates from broken pairs mapping to pRW1p were then extracted. Broken pair mates occur when two paired end reads do not map to adjacent locations on the reference genome; broken pair mates collected from reads mapping to pRW1p DNA should map to regions adjacent to the site of insertion. Because of sequence similarity between the constructs used to create donor and recipient strains, insertions in both strains can be found using this approach. For each progeny strain genome sequenced, around half of the broken pair mates mapped to supercontig 15 of *Fol*4287 that is located on chromosome 10 close to position 8 53 000 (www.broadinstitute.org). This region is homologous to a region located on supercontig 8 close to position 14 46 000 in *Fo*47. Since this insertion is shared by all three progeny strains tested, but not present in donor C, it has to originate from the recipient strain. From this we conclude that the *HPH* marker in the recipient strain is located on chromosome 8 of *Fo*47 (which includes supercontig 8 and 11).

Since all progeny strains contain chromosome 14 from *Fol*4287, we expected pRW1p to be inserted into this chromosome in all donor strains. Indeed, donor C and progeny strain c1 have the *BLE* construct in chromosome 14, with broken pair mates for the plasmid primarily mapping close to position 22 8 000 on supercontig 36. This location fits with the frequent production of double resistant progeny, similar to the amount produced when testing chromosome 14 for transfer in the targeted approach. However, although CHEF gel and PCR analysis clearly showed the presence of chromosome 14 in all progeny strains from co-incubations of both donor A and B with the recipient, the broken pair mates for the insertions (the ones unique to each strain) did *not* map to chromosome 14; in a1 the insertion mapped to supercontig 13 (close to position 81 7 000) and in b1 to supercontig 3 (close to position 31 48 000). These positions are located on chromosome 7 and 8 respectively.

To determine whether these two core chromosomes of the *Fol*4287 genome had indeed been transferred to the recipient strain, along with chromosome 14, the mapping results were further analyzed. Transfer of any region of the *Fol*4287 genome to the *Fo*47 background can be identified based on the presence of single nucleotide polymorphisms (SNPs) between core chromosomes of these two strains. By mapping reads with 100% identity and 100% coverage to *Fol*4287, all reads originating from the recipient genome containing SNPs compared with the donor are discarded. The resulting *Fol*4287 unique read mappings were visualized by calculating the average read depth in 10-Kb windows and subsequently by mapping them to the genome assembly. In Fig. 3, the results are visualized. It is clear that all three progeny strains carry all chromosome 14 sequences (Fig. 3, blue arrows), confirming transfer of the entire chromosome 14. There are no clear indications for duplicated regions that could explain the larger size of chromosose 14 in donor C. No other peculiarities were found in progeny strain c1, indicating simply transfer of (enlarged) chromosome 14, but no other events. Strains a1 and b1, with the marker located on core chromosome 7 or 8 of their respective donor strains, indeed showed the presence of the respective chromosomal sequences of *Fol*4287 (Fig. 3A and B, lower panels). In strain a1, a clear increase in average read depth after mapping to the *Fol*4287 genome was found specifically for chromosome 7. Interestingly, not all of chromosome 7 from *Fol*4287 appears to be present (Fig. 3, strain a1 lower panel), but rather about one third of this chromosome. The region that is present is approximately 1.6 Mb in length and consists of supercontig 13 excluding the first 76 020 bp (not visible in Fig. 3). This region of chromosome 7 of *Fol*4287 is homologous to parts of supercontig 6 of *Fo*47, which is

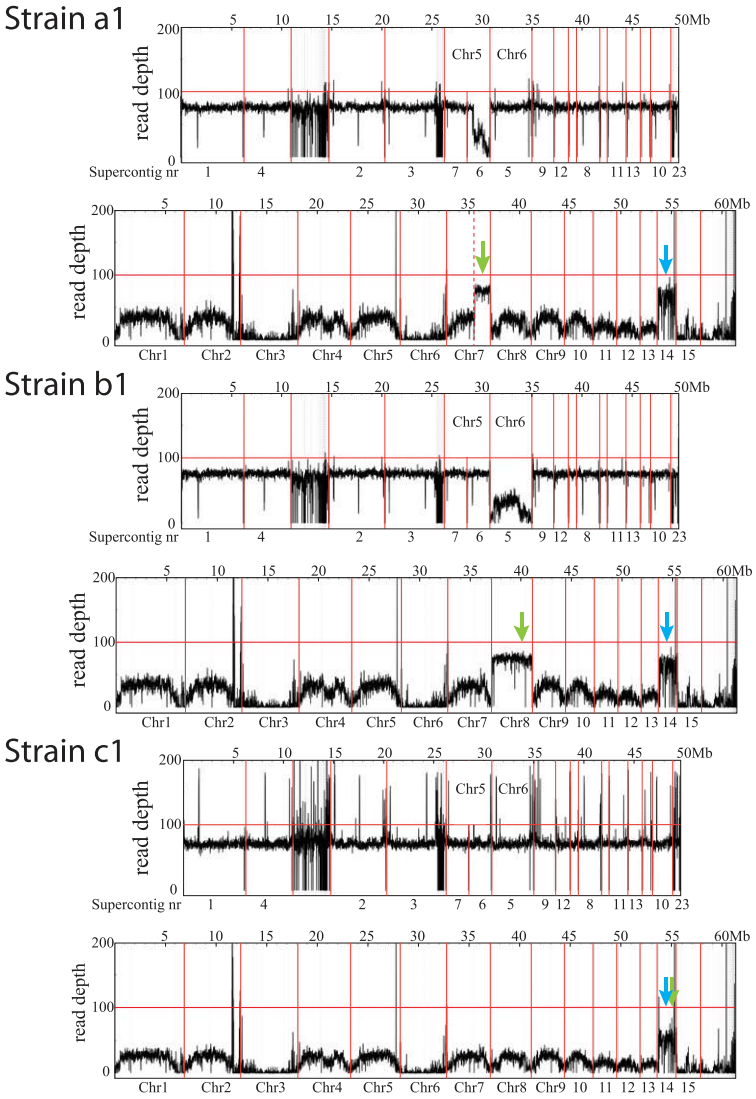

Fig. 3. Average read depth plots for recipient strains show transfer of two core chromosomes as well as of chromosome 14. The genomes of progeny strains a1, b1 and c1 were sequenced and mapped to both the *Fo*47 and *Fol*4287 reference genomes. To visualize these mappings average read counts in 10Kb windows are calculated and displayed on the *Y*-axis while the *x*-axis depicts chromosomes for mappings to the *Fol*4287 genome and major supercontigs for mappings to *Fo*47 as well as the length of the genome in Mb (on top). Approximate positions of the marker in the donor strains are depicted with a green arrow in their respective mappings to *Fol*4287. In strain a1 supercontig 13 from chromosome 7 from *Fol*4287 is present, while part of the homologous supercontig 6 of chromosome 5 of Fo47 is missing. Strain b1 received all of chromosome 8, which comprises almost all of chromosome 6 from *Fo*47, which is no longer present. Both these progeny strains also received chromosome 14 indicated with blue arrows. In the case of c1 this is the only material present from *Fol*4287. No parts of the *Fo*47 genome are missing in this progeny strain. Chromosome 14 of *Fol*4287 does not have a homologous region in *Fo*47. In general, large peaks or dips in the average read count are a consequence of missasigned repetitive sequences and differences between the reference assembly and our biological samples.

located on chromosome 5 in that strain. Surprisingly, when reads are mapped to the *Fo*47 reference genome, most of supercontig 6 appeared to be missing from strain a1, indicating loss of the homologous region in the recipient genome. The first 600 Kb of the ~2.2-Mb long supercontig is still present, leaving a deletion of around 1.6 Mb. There are no repetitive elements present at the borders of the transferred region or at the borders of the lost regions.

Progeny strain b1 showed gain of chromosome 8 from *Fol*4287, which is approximately 4 Mb long and appears to have been transferred completely (Fig. 3, middle green arrow). Chromosome 8 of *Fol*4287 is homologous to supercontigs 5 and 19 from *Fo*47. Both are missing when the reads of recipient b1 are mapped to the *Fo*47 genome (Fig. 3B upper panel; supercontig 19 not indicated). Supercontig 5 and 19 of *Fo*47 together make up chromosome 6, so it appears in this case that the entire chromosome 6 from *Fo*47 is absent in the progeny strains. These data suggest that transfer of (parts of) a core chromosome from *Fol*4287 to *Fo*47 is accompanied by loss of the corresponding chromosome or region in the recipient. The gain of core regions from the donor and the loss of homologous regions in the recipient suggest an exchange of homologous sequences.

All individual progeny strains were tested by PCR for the presence of *Fol*4287 chromosome 7 (a1–a4) or chromosome 8 (b1, b2) sequences or for the presence of the homologous regions in *Fo*47 with sets of primers that differentiate between these two strains based on an insertion of the *Foxy* retrotransposon (Ma *et al.*, 2010). Two markers were used per chromosome, each located on a different supercontig. For chromosome 7 the markers are located at position 513.000 on supercontig 5 and 43 6 000 on supercontig 13; for chromosome 8 markers are located at 36 7 000 and 19 22 000 on supercontig 3. For progeny strains a1–a4 it was found that in all four cases the marker corresponding to supercontig 13 originated from *Fol*4287 and the one corresponding to supercontig 5 from *Fo*47,

indicating partial transfer of chromosome 7 in all strains. In strains b1 and b2 both markers tested were present, indicating transfer of the full chromosome 8 or at least a large part thereof. In conclusion, in all cases gain of a region from *Fol*4287 led to the loss of the homologous region from the recipient strain.

Non-pathogenic strains gain pathogenicity after transfer of chromosome 14 from Fol4287

Transfer of chromosome 14 from the pathogenic strain *Fol*007 to the non-pathogenic strain *Fo*47 confers pathogenicity to the recipient (Ma *et al*., 2010). We investigated whether *Fo*47 also gains pathogenicity after acquiring chromosome 14 from the reference strain, *Fol4287*. While some variation is present between the three donor strains, each of these is fully virulent with most plants showing severe symptoms or complete wilting (Fig. 4A and B). As expected, plants infected with strain *Fo47* and the mock-inoculated plants produced no disease symptoms (Fig. 4A *Fo*47, 4B). In contrast, all of the progeny strains tested produced disease symptoms. Compared with the pathogenic donor and control strains, progeny strains showed an intermediate phenotype, mostly scoring a relatively low disease index; in many cases only one brown vessel was found in the hypocotyl of the plants while their general appearance was still quite normal. While severe symptoms or complete wilting were rare, so was a complete absence of symptoms, indicating a consistent gain of pathogenicity with moderate virulence (Fig. 4A and B). These low levels of virulence had only a moderate or no effect on overall plant weight when compared with *Fo47* (Fig. 4C). Infection with the donor strains did lead to a clear reduction in plant weight (Fig. 4C). In conclusion, transfer of chromosome 14 from any of the three *Fol*4287-derived donor strains, including donor C with a larger version of chromosome 14, leads to gain of pathogenicity with moderate to low levels of virulence by the initially non-pathogenic recipient strain.

Discussion

The aim of this study was to obtain more insight into the processes underlying HCT and possible restrictions in chromosome size or other properties. We show that exchange of two core chromosomes or parts thereof is possible, albeit at a low frequency. Interestingly, transfer of chromosome 14 always accompanied exchange of (part of) core chromosomes. Transfer of other core and LS chromosomes of similar size was not observed. From this we conclude that transfer is not restricted to LS chromosomes and size is not the determining factor.

We reached this conclusion by targeting the large chromosome 1 and the small chromosomes 12, 14 and 15 for transfer. Chromosome 12 is a core chromosome (with homologs in other *Fusarium* species), while chromosome 14 and 15 are LS. Of these chromosomes, only chromosome 14 was found to be transferred to the non-pathogenic recipient. While chromosome 1 could be excluded based on size, chromosome 12 is quite similar in size to chromosome 14 (2.4 Mb and 2 Mb respectively). We did not observe transfer of LS chromosome 15, which suggests that a propensity for horizontal transfer is not a property of all LS chromosomes. Co-transfer of the smallest chromosome from *Fol*007 (which is also LS but not homologous to chromosome 15 of *Fol*4287) has been observed previously (Ma *et al*., 2010). Apparently, properties particular to LS chromosomes facilitate but are not sufficient for transfer.

He *et al*. suggested two possible pathways for transfer of chromosomes: uptake through nuclear pores or nuclear fusion followed by selective degradation (He *et al*., 1998). Our observations provide some insight into which pathway is more likely. In *F. oxysporum*, breakdown of nuclei was observed following hyphal fusion and migration of nuclei, in vegetatively compatible strains (Ruiz-Roldan *et al*., 2010). This results in one cellular compartment containing both an intact nucleus and any residual material from the degraded nucleus. Potentially this could be followed by uptake of genetic material by the surviving nucleus. However, our results rather suggest that nuclear fusion followed by selective loss of chromosomes from one of the fusion partners is more likely – even though the mechanism of such selective loss remains elusive. We show that part of a chromosome (chromosome 7 of *Fol*4287) can be exchanged, which suggests homologous recombination. We also observed co-transfer of chromosome 14 in all cases where transfer of a core region was selected for. If transfer requires nuclear fusion, all of the donor and recipient chromosomes would be in the same nucleus at least transiently, which would explain exchange of (large) chromosomes, recombination and gain of several chromosomes in a single event. Although this process shares mechanistic features with the parasexual cycle which has previously been described between compatible strains of *F. oxysporum* (Buxton, 1956; Teunissen *et al*., 2002) the outcome is very different. In a parasexual cycle, which was forced by either combining auxotrophs (Buxton, 1956) or following protoplast fusions (Teunissen *et al*., 2002), segregants possess a mixture of genetic material and properties from both parents. In transfer and exchange no such massive rearrangements between genomes of non-compatible strains takes place, as evidenced by CHEF gel, sequencing and marker analysis of several ‘c’ progeny (data not shown).

While we demonstrate that transfer of core regions is possible, the observed frequency of exchange for core chromosomes is much lower than for transfer of LS chromosome 14, especially when selected for. Transfer of this

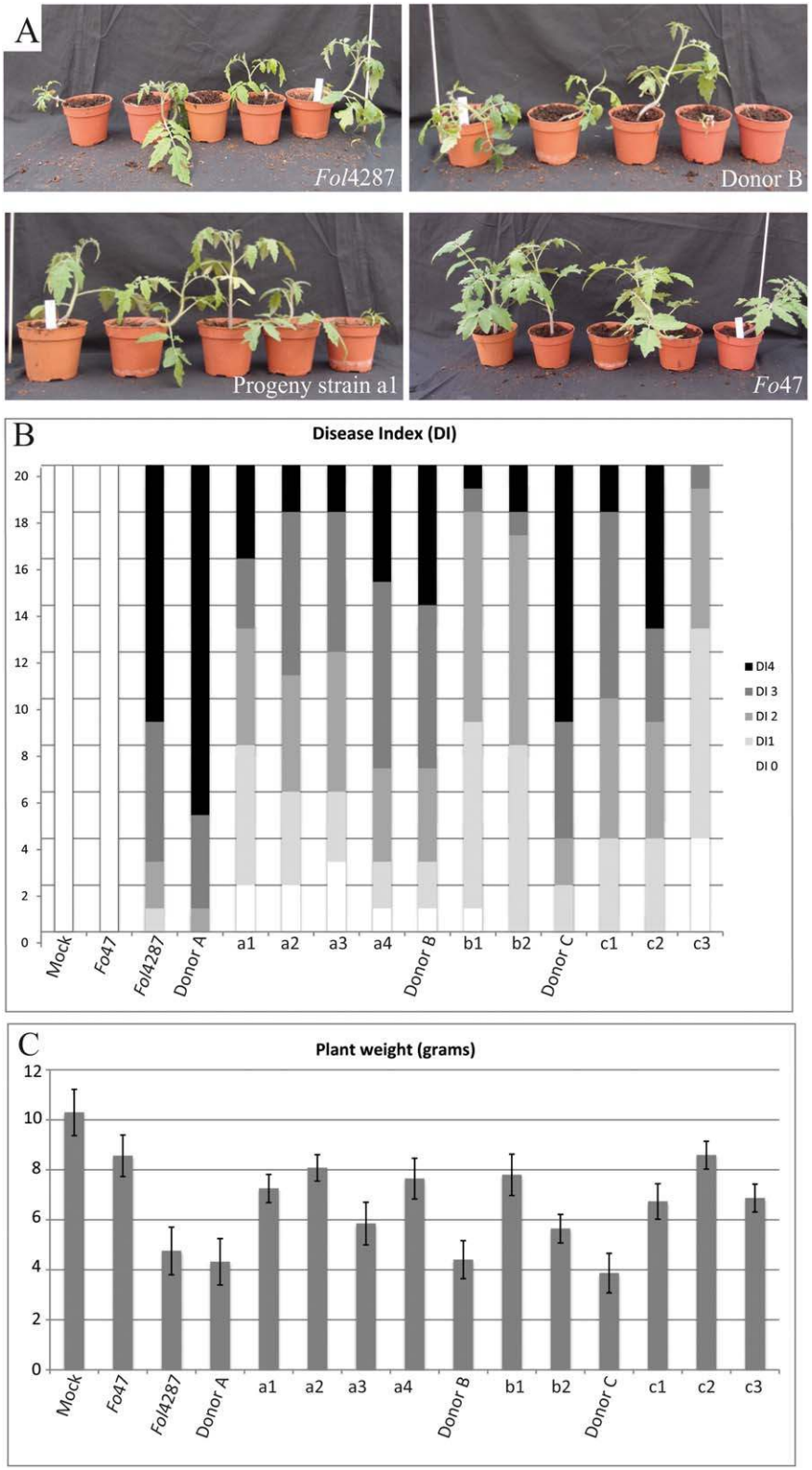

Fig. 4. A bioassay with donor and recipient strains shows gain of pathogenicity with gain of chromosome 14.
A. Representative pictures of plants infected with wild-type *Fol*4287, one of the pathogenic donors and one of the progeny strains having gained chromosome 14. The wild-type and donor strains show mostly severely diseased plants while the progeny strains have some plants showing clear symptoms while others appear healthy.
B. Disease index for all donor and their progeny strains. Disease index is scored from 0 to 4 based on the number of brown vessels and macroscopic effects. The *Y*-axis indicates the number of plants while the colour indicates the disease index. This experiment was repeated with similar results. All donors cause disease to the same extent as the wild-type strain. While disease index caused by progeny strains is usually low, often without macroscopic effects but with one or two brown vessels, almost all plants show symptoms. This confirms that gain of chromosome 14 leads to gain of pathogenicity.
C. Average plant weight after infection. Similar to the disease index, infection with donor strains leads to a strong reduction in weight compared with mock inoculations indicating full pathogenicity. The progeny strains show a much milder or no reduction in weight, confirming gain of pathogenicity with moderate virulence.

chromosome also occurs when not selected for (i.e. when the marker is inserted in chromosome 7 or 8). Possibly, chromosome 14 has features that make it especially amenable for transfer and that are not shared even with other LS chromosomes. Transfer capability (which may in fact mean resistance to elimination in a transient diploid state)

could be the result of organization of the DNA within the nucleus or other not yet defined features. For core chromosomes we observe exchange of homologous sequences – possibly elimination requires the presence of a homologous chromosome. The level of macrosyntenty may determine exchange of core chromosomes, meaning transferability is a result of an interplay between donor and recipient genomes. If this is the case, different combinations of donor and recipient strains would yield different results. Since chromosome 14 shares no homology with the recipient genome it could thereby evade degradation. However, this leaves the lack of transfer of other LS regions unexplained. An alternative explanation is the potential presence of some factor encoded on chromosome 14 which facilitates transfer (or rather protects it from elimination) as suggested previously (Rosewich and Kistler, 2000). Currently, strains lacking chromosome 14 have been generated in our lab, providing the means to differentiate between these two options. Assuming that a transient diploid state is the most likely intermediate for HCT, it is remarkable that we never see aneuploidy, i.e. the stable presence of two homologous chromosomes. In *Mycosphaerella graminicola* aneuploidy of both core and LS chromosomes has been observed after meiosis (a process that has not been observed in *F. oxysporum)* (Goodwin *et al*., 2011). The absence of aneuploidy in *F. oxyporum* in our observations could either result from a directed elimination process of one set of chromosomes or an inability to stably maintain aneuploidy.

Surprisingly, only one marker insertion was found on each of the chromosomes 7, 8 and 14 among 269 transformants, which is much less than expected assuming random insertion of T-DNA in the genome. The low number of strains found capable of marker transfer might simply be the result of lack of sensitivity of our experimental setup. Potentially, LS chromosomes could be more or less likely sites of insertion for the marker.

The fact that strain c1 with the marker insertion in chromosome 14 contained a larger version of chromosome 14 remains puzzling. While the event that led to the increase in size might have been (in part) induced by insertion of the marker, the identity of the extra material could not be determined by genome sequencing. The method of mapping the average read depth to the reference genome has been used successfully before to find both deletions and duplications (Urban *et al*., 2015; our observations, submitted). Why this was not successful with this strain remains unclear, but it might be the result of repetitive sequences present in the LS part of the genome.

While interesting new questions arise from the results presented in this paper, a number of longstanding questions have been answered. Even though it is not as common as transfer of the *Fol* pathogenicity chromosome, exchange of core chromosomes between incompatible strains is possible. How uptake of a core chromosome leads to the loss of homologous regions of the recipient remains to be discovered. Also, why chromosome 14 in particular is commonly transferred remains unclear. What is clear, however, is that the transfer of large chromosomes and the co-migration of several chromosomes during the same event takes place, suggesting that transfer results from nuclear fusion and selective chromosome loss rather than uptake of chromosomes by the recipient nucleus.

Experimental procedures

Strains and plasmids used

Strain *F. oxysporum* f. sp. *lycopersici* 4287 (*Fol*4287) (Di Pietro and Roncero, 1996) was transformed with pRW1p (Houterman *et al*., 2008) to create 269 individual transformants used in a screen for transfer of the *BLE* resistance gene present in pRW1p. To obtain strains with a fusion of the *HPH* hygromycin resistance gene and *GFP* (*HPH-GFP*) on chromosomes of interest, *Fol*4287 was transformed according to Mullins *et al*. (2001) with p12HG, p14HG or p15HG. Each of these constructs was made by cloning two ~1-Kb fragments, amplified from *Fol*4287 genomic DNA, into the multiple cloning site of pPK2-HPH-GFP (Michielse *et al*., 2009) such that homologous recombination between the plasmid and the genome results in insertion of the *HPH-GFP* fusion gene at the desired site without deleting any material. The primers used as well as the chromosomal locations targeted are given in Supporting Information Table S1. Each location has a forward and reverse primer for the left flank (LF and LR) and right flank (RF and RR). Insertion at the correct site is tested by using primers just outside the left and right flanks (LT and RT) combined with primers on the backbone (LB_R and RB_F). The resulting strains were called 12HG, 14HG and 15HG. The strain used as a recipient in the screen was *Fo*47H1 (Ma *et al*., 2010) which is *Fo*47 (Lemanceau and Alabouvette, 1991) transformed with pPK2-HPH-GFP (Michielse *et al*., 2009). *Fo*47 transformed with pGRB (Vlaardingerbroek *et al*., 2015) was used as a recipient in the experiments with 12HG, 14HG1, 14HG2 and 15HG as potential chromosome donor strains. Two individual transformants, *Fo*47GRB1 and *Fo*47GRB2, were used.

Transfer experiments

Chromosome transfer was performed with an adapted version of the method described previously (Ma *et al*., 2010). Donor and recipient strains were grown in minimal liquid medium containing 3% sucrose, 0.17% yeast nitrogen base and 100 mM KNO_3 in a shaker (175 rpm) at 25°C for 5 days. Spores were filtered through a double layer of miracloth (Merck) and spore concentration estimated using a haemocytometer. Two times 10^5 spores of each strain were co-incubated on either CDA (Oxoid) or PDA (BD biosciences) for 5 days before collecting spores from the plate by scraping using 2 ml of milli-Q filtered water. One millilitre of this spore suspension was transferred to CDA containing 0.1 M Tris pH 8

supplemented with 100 μg/ml hygromycin (Duchefa) and 100 μg/ml zeocin (Invitrogen). In the targeted approach, double drug resistant colonies were picked after 3–5 days and transferred to a plate containing only zeocin to confirm zeocin resistance. These colonies were monospored on new plates containing zeocin after which DNA was isolated for confirmation of the transfer of markers. In the screen, colonies were picked after 2–3 days rather than 5 to reduce false positives, and subsequently transferred to plates containing zeocin only, to select against zeocin sensitive (i.e. false positive) colonies (which emerge eventually during dual selection). Markers used for effector genes and for the supercontigs of *Fol4287* are displayed in Supporting Information Table S2.

CHEF gels

Preparation of protoplasts and running of CHEF gels was performed as described previously (Teunissen *et al*., 2002; 2003). Liquid cultures of *F. oxysporum* strains (liquid medium containing 0.17% yeast nitrogen base, 100 mM KNO_3 and 3% sucrose) were grown in flasks (250 ml) for 1 week, after which conidia were collected by filtration through two layers of miracloth (Merck) and the concentration estimated using a haemocytometer. 5×10^8 spores per culture were transferred to 40-ml PDB (BD Biosciences). After incubating for 12–16 h the fresh mycelium was collected and incubated at 25°C for 12–16 h with 50 mg/ml Glucanex (Sigma) and 5 mg/ml Driselase (Sigma) in sorbitol solution (1 M sorbitol, 50 mM $CaCl_2$, 10 mM Tris-HCl pH 7.4). Protoplasts were collected by centrifugation and cast in plugs at a concentration of 1.5×10^8 protoplasts per ml. Chromosomes were separated by running for 10 days in Seakem agarose (Lonza) at 1.6 V/cm in a CHEF-DRII system (Biorad) at 5°C with switch times between 1200 and 4800 s. For visualization of DNA, gels were stained with Ethidium Bromide and de-stained using 0.5 xTBE.

Disease assays

Disease assays were performed as described previously (Rep *et al*., 2004). Spores were collected from *F. oxysporum* cultures grown for 5 days in PDB (BD Biosciences). Spores were filtered through two layers of miracloth, washed, the concentration estimated using a haemocytometer and then diluted to a final concentration of 10^7 spores per ml. Ten-day-old C32 tomato seedlings were uprooted, the roots clipped, dipped in the spore suspension and replanted in individual pots. After 3 weeks the part of the plant above the cotyledons was cut off and weighed and disease index was scored based on vessel browning from 0 to 4 (0 no brown vessels at the cotyledons or just above the soil; 1 one brown vessel at the cotyledons or just above the soil; 2 two brown vessels; 3 more than two brown vessels; 4 all vessels brown, severe growth defects or completely dead plants).

Genome sequence analysis

Strains to be sequenced were grown in liquid medium (0.17% yeast nitrogen base, 10 mM KNO3 and 3% sucrose) for 5 days, mycelium was collected and freeze dried. DNA was isolated from the mycelium by phenol-chloroform extraction. Library construction and Illumina sequencing was performed by Keygene NV (Wageningen, the Netherlands). An average coverage of at least 50X was achieved with 125 bp paired-end reads. Mapping of these reads to the reference genomes (Fusarium Comparative Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)) was performed using CLC genomics workbench 8.5.1. Reads were trimmed (5' end 15nt) to remove adapter sequences. Reads were mapped to the *Fol*4287 or the *Fo*47 genome only if coverage and identity were 100%, to differentiate between these two strains based on SNPs. For visualization of the read counts the mapped reads were sorted using SAMtools sort function. Duplicate reads were removed using Picard Tools with standard settings. Ten kb non-overlapping sliding windows are displayed.

Acknowledgements

This work was made possible by a Vici grant from the Netherlands Organization for Scientific Research (NWO) (to M.R.).

References

Akagi, Y., Akamatsu, H., Otani, H., and Kodama, M. (2009) Horizontal chromosome transfer, a mechanism for the evolution and differentiation of a plant-pathogenic fungus. *Eukaryot Cell* **8:** 1732–1738.

Buxton, E.W. (1956) Heterokaryosis and parasexual recombination in pathogenic strains of *Fusarium oxysporum*. *Microbiology* **15:** 133–139.

Connolly, L.R., Smith, K.M., and Freitag, M. (2013) The *Fusarium graminearum* histone H3 K27 methyltransferase KMT6 regulates development and expression of secondary metabolite gene clusters. *PLoS Genet* **9:** e1003916.

Covert, S.F. (1998) Supernumerary chromosomes in filamentous fungi. *Curr Genet* **33:** 311–319.

Croll, D., and McDonald, B.A. (2012) The accessory genome as a cradle for adaptive evolution in pathogens. *PloS Pathog* **8:** e1002608.

De Souza, C.P., and Osmani, S.A. (2007) Mitosis, not just open or closed. *Eukaryot Cell* **6:** 1521–1527.

Di Pietro, A., and Roncero, M.I.G. (1996) Endopolygalacturonase from *Fusarium oxysporum* f. sp. *lycopersici*: purification, characterization, and production during infection of tomato plants. *Phytopathology* **86:** 1324–1330.

Goodwin, S.B., M'Barek, S.B., Dhillon, B., Wittenberg, A.H., Crane, C.F., Hane, J.K., et al. (2011) Finished genome of the fungal wheat pathogen Mycosphaerella graminicola reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. *Plos Genet* **7:** e1002070.

Han, Y., Liu, X., Benny, U., Kistler, H.C., and VanEtten, H.D. (2001) Genes determining pathogenicity to pea are clustered on a supernumerary chromosome in the fungal plant pathogen *Nectria haematococca*. *Plant J* **25:** 305–314.

He, C., Rusu, A.G., Poplawski, A.M., Irwin, J.A., and Manners, J.M. (1998) Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the fungus *Colletotrichum gloeosporioides*. *Genetics* **150:** 1459–1466.

Houterman, P.M., Speijer, D., Dekker, H.L., de Koster, C.G., Cornelissen, B.J.C., and Rep, M. (2007) The mixed xylem

sap proteome of *Fusarium oxysporum*-infected tomato plants. *Mol Plant Pathol* **8:** 215–221.
Houterman, P.M., Cornelissen, B.J., and Rep, M. (2008) Suppression of plant resistance gene-based immunity by a fungal effector. *PloS Pathog* **4:** e1000061.
Ishikawa, F.H., Souza, E.A., Shoji, J., Connolly, L., Freitag, M., Read, N.D., and Roca, M.G. (2012) Heterokaryon incompatibility is suppressed following conidial anastomosis tube fusion in a fungal plant pathogen. *PloS One* **7:** e31175.
Lemanceau, P., and Alabouvette, C. (1991) Biological control of fusarium diseases by fluorescent *Pseudomonas* and non-pathogenic *Fusarium*. *Crop Prot* **10:** 279–286.
Ma, L., van der Does, H.C., Borkovich, K.A., Coleman, J.J., Daboussi, M., Di Pietro, A., *et al*. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464:** 367–373.
Manners, J.M., and He, C. (2011) Slow-growing heterokaryons as potential intermediates in supernumerary chromosome transfer between biotypes of *Colletotrichum gloeosporioides*. *Mycol Prog* **10:** 383–388.
Mehrabi, R., Bahkali, A.H., Abd-Elsalam, K.A., Moslem, M., Ben M'Barek, S., Gohari, A.M., *et al*. (2011) Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. *FEMS Microbiol Rev* **35:** 542–554.
Michielse, C., van Wijk, R., Reijnen, L., Cornelissen, B.J.C., and Rep, M. (2009) Insight into the molecular requirements for pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* through large-scale insertional mutagenesis. *Genome Biol* **10:** R4.
Mullins, E.D., Chen, X., Romaine, P., Raina, R., Geiser, D.M., and Kang, S. (2001) Agrobacterium-mediated transformation of *Fusarium oxysporum*: an efficient tool for insertional mutagenesis and gene transfer. *Phytopathology* **91:** 173–180.
Raffaele, S., and Kamoun, S. (2012) Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat Rev Microbiol* **10:** 417–430.
Rep, M., Van Der Does, H.C., Meijer, M., Van Wijk, R., Houterman, P.M., Dekker, H.L., *et al*. (2004) A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for I 3mediated resistance in tomato. *Mol Microbiol* **53:** 1373–1383.
Rosewich, U.L., and Kistler, H.C. (2000) Role of horizontal gene transfer in the evolution of fungi 1. *Annu Rev Phytopathol* **38:** 325–363.
Ruiz-Roldán, M.C., Köhli, M., Roncero, M.I.G., Philippsen, P., Di Pietro, A., & Espeso, E.A. (2010). Nuclear dynamics during germination, conidiation, and hyphal fusion of Fusarium oxysporum. *Eukaryotic cell* **9:** 1216–1224.
Temporini, E.D., and VanEtten, H.D. (2004) An analysis of the phylogenetic distribution of the pea pathogenicity genes of *Nectria haematococca* MPVI supports the hypothesis of their origin by horizontal transfer and uncovers a potentially new pathogen of garden pea: *Neocosmospora boniensis*. *Curr Genet* **46:** 29–36.
Teunissen, H.A.S., Rep, M., Houterman, P.M., Cornelissen, B.J.C., & Haring, M.A. (2003). Construction of a mitotic linkage map of Fusarium oxysporumbased on Foxy-AFLPs. *Molecular Genetics and Genomics* **269:** 215–226.
Teunissen, H., Verkooijen, J., Cornelissen, B., and Haring, M. (2002) Genetic exchange of avirulence determinants and extensive karyotype rearrangements in parasexual recombinants of *Fusarium oxysporum*. *Mol Genet Genomics* **268:** 298–310.
Urban, M., King, R., Hassani-Pak, K., and Hammond-Kosack, K.E. (2015) Whole-genome analysis of Fusarium graminearum insertional mutants identifies virulence associated genes and unmasks untagged chromosomal deletions. *BMC Genomics* **16:** 261.
Vlaardingerbroek, I., Beerens, B., Shahi, S., and Rep, M. (2015) Fluorescence Assisted Selection of Transformants (FAST): using flow cytometry to select fungal transformants. *Fungal Genet Biol* **76:** 104–109.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Markers for chromosome 14 but no other LS regions are present in progeny strains. Progeny strains that were not sequenced were tested for transfer of LS regions of the genome from the donor to the recipient. Primers were used targeting specific insertions of the *Foxy* transposon on LS chromosomes of *Fol*4287. Location on chromosome and supercontig are indicated. Only bands for chromosome 14 are visible. *Fo*47 was included as negative and *Fol*4287 as positive control.
Fig. S2. Markers for chromosome 14 are present in double resistant progeny strains.
A. Schematic representation of chromosome 14 with the location of the genes encoding for small proteins Secreted in Xylem (*SIX*) and *ORX1* as well as the location of the primers targeting insertions of the *Foxy* transposon.
B. PCR fragments for genes used as markers for chromosome 14 in all progeny strains resulting from co-incubation of donors A, B and C with *Fo*47H1. Although amplification of *SIX2* was not always successful all progeny strains contain markers spread over the chromosome indicating transfer of large parts of the chromosome (including known effector genes) from donor to progeny strains.
Table S1. Primers used for cloning.
Table S2. Markers for chromosomes.