Research Article

Constructing Physical and Genomic Maps for *Puccinia striiformis* f. sp. *tritici*, the Wheat Stripe Rust Pathogen, by Comparing Its EST Sequences to the Genomic Sequence of *P. graminis* f. sp. *tritici*, the Wheat Stem Rust Pathogen

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The wheat stripe rust fungus, *Puccinia striiformis* f. sp. *tritici* (*Pst*), does not have a known alternate host for sexual reproduction, which makes it impossible to study gene linkages through classic genetic and molecular mapping approaches. In this study, we compared 4,219 *Pst* expression sequence tags (ESTs) to the genomic sequence of *P. graminis* f. sp. *tritici* (*Pgt*), the wheat stem rust fungus, using BLAST searches. The percentages of homologous genes varied greatly among different *Pst* libraries with 54.51%, 51.21%, and 13.61% for the urediniospore, germinated urediniospore, and haustorial libraries, respectively, with an average of 33.92%. The 1,432 *Pst* genes with significant homology with *Pgt* sequences were grouped into physical groups corresponding to 237 *Pgt* supercontigs. The physical relationship was demonstrated by 12 pairs (57%), out of 21 selected *Pst* gene pairs, through PCR screening of a *Pst* BAC library. The results indicate that the *Pgt* genome sequence is useful in constructing *Pst* physical maps.

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1. Introduction

*Puccinia striiformis* f. sp. *tritici* (*Pst*) is the causal agent of stripe rust, one of the most important diseases on wheat in many countries of the world [1, 2]. The disease is a major constraint to wheat production and is a serious threat to the global food security. Although the disease is economically important, only limited studies on the genome and functional genomics of the fungal pathogen have been reported [3–6]. This is an obstacle to our understanding of the pathogen’s evolution, especially changes of virulence that often overcome resistance in wheat cultivars [1, 2, 7, 8].

*Pst* is an obligate biotrophic fungus that completely depends upon its host plants for continuing growth and reproduction. Techniques for transformation, gene knockout, and transient expression are still to be developed. This excludes the use of molecular techniques, such as restriction enzyme-mediated insertional mutagenesis and gene transformation.

Unlike *P. graminis* f. sp. *tritici* (*Pgt*, the wheat stem rust pathogen) and *P. triticina* (*Pt*, the wheat leaf rust pathogen), *Pst* is a microcyclic rust fungus and has only three spore stages, urediniospore, teliospore, and basidiospore, and does not have known pycniospore and aeciospore stages [1, 2]. Because of the lack of the pycnial sexual stage and alternate host for sexual reproduction, it is impossible to study *Pst* genes through a classic genetic approach and map-based cloning. Thus, gene organization and physical relationships could not be studied for *Pst* using the molecular mapping approach.

A physical map is useful for studying genome structures, determining gene organization, identifying important genes, and comparing related species for understanding evolutionary-ary relationships. The discovery of conserved chromosomal segments between humans and animals in 1984 [9] led later to the construction of physical maps for human and mouse [10–13]. Interestingly, comparative gene mapping reveals...
that chicken, a nonmammalian vertebrate, has conserved
genome sequence synten with humans [14, 15]. Compara-
tive genome approaches have also been widely used to study
related species in plants [16–19] and fungi [20–23]. These
studies demonstrate that comparative genomic analysis is
a powerful approach for studying genomes and genes in
organisms that are hard to study using traditional genetic
approaches.

Recently, several genetic libraries for \( Pst \) have become
available, including a BAC library [3], a full-length cDNA
library from urediniospores [4], germinated urediniospore
or germ-tube EST library [5], and a haustorial EST library
[6]. A total of more than 15,000 ESTs were sequenced,
from which 4,219 unisequences were characterized and
their putative functions were identified through sequence
comparison with other fungal genes in GenBank databases.
However, the physical and genetic relationships of these
genes have not been determined. Since \( Pst \) genome sequenc-
ing has just been started, here we have used the available
\( Pgt \) genome sequence (http://www.broadinstitute.org/anno-
tation/genome/puccinia_group/MultiHome.html) for con-
structing physical maps for \( Pst \) genes. The study was based on
the assumption that \( Pst \) and \( Pgt \) share considerable sequence
homology and genome synten. The specific objectives of
this study were to (1) determine the homology of \( Pst \)
EST unisequences to \( Pgt \) genomic sequences, (2) construct
physical maps for the \( Pst \) genes using the \( Pgt \) sequences
as the references, and (3) verify the physical relationships
of selected \( Pst \) genes using PCR screening of the \( Pst \) BAC
library. Although much of the physical relationship needs to
be verified by whole-genome sequence, the physical maps
generated in this study should provide a basic framework
for assisting \( Pst \) sequence assembling and gene annotation
with \( Pgt \) sequences and also should be useful for localizing
functional genes, positional cloning of full-length genes,
and generating information about exons and introns for \( Pst \)
genes.

2. Materials and Methods

2.1. Data. Genome-based EST mapping requires the genome
map and transcript sequences. The three \( Pst \) cDNA
libraries were generated from three different growing stages,
urediniospores (Ured), germinated urediniospores (Ger-
mUred)/germ tubes, and haustoria (Haus). The Ured and
Haus cDNA libraries were constructed from mRNA of PST-
78, a typical US race [4, 6], and the GermUred library
was from mRNA of CYR32, a typical Chinese race [5]. A
total of 4,219 unisequences, which were obtained from more
than 15,000 clones sequenced from the three libraries after
removing sequences of poor quality (<100 bp inserts) and
repetitions and forming contigs (4, 5, 6, Chen and associates,
unpublished), were used in this study for comparing with the
\( Pgt \) genomic sequence. The \( Pst \) genome sequence was
downloaded from the NCBI Genome Project Puccinia grami-
nis Database (http://www.broad.mit.edu/annotation/geno-
me/puccinia_graminis), consisting of 392 genome supercon-
tigs and 4,775 contigs.

2.2. Mapping \( Pst \) EST Sequences against the \( Pgt \) Genome
Sequence. All \( Pst \) ESTs were mapped against the \( Pgt \) genome
using the BLASTN program [24]. We used the high-speed
service computer system of the Washington State University
Bioinformatics Center for BLAST and homology searches.
The \( Pgt \) genome and \( Pst \) EST sequences were transferred to
a server computer using the SSH (Secure Shell) software as
fasta format files. Sequences of low homologous alignment
were filtered out using the e value of 1.00E-5 as a cut point.
The alignable ESTs were assembled according to the 4,775
contigs in the 392 supercontigs of the \( Pgt \) genome sequence.
Detailed alignment information was edited in an Excel file.
To see the positions of the \( Pst \) ESTs corresponding to the
\( Pgt \) genome, physical maps were constructed. Physical maps
containing \( Pst \) supercontigs illustrated the physical
position order of the genes, length of each EST, and the
distances between genes. The genes localized in a single contig
were marked using a sign of “[ ]” and the alignment start and
end positions of the \( Pgt \) genome were given in parentheses.
Because the ESTs were transcribed from the genome and
the introns were spliced after alternative splicing, the ESTs
represent the exon sequences. Therefore, it was important
that we were able to get the information about the alternative
gene splicing and the intron number from the maps. If a \( Pst \)
EST sequence was aligned to a location in the \( Pgt \) genome
as a series of fragments, these genes were likely to show
alternative splicing, and the number of exons was marked
after the parentheses on the map. All sketch maps of \( Pst \)
genomes are shown in file 1 in Supplementary Material available
online at doi: 10.1155/2009/302620.

2.3. Verification of Physical Relationships of Selected \( Pst \) Genes.
Although \( Pgt \) is most closely related to \( Pst \) among the fungi
whose whole genome has been sequenced so far, their gene
sequences and locations could be different for some genes. To
validate the veracity of the alignment, we selected 42 genes
as 21 pairs. The sequences of the 42 genes were used to
design primers. The 42 primer pairs (Table 1) were used to
amplify BAC clones. If a single BAC clone was amplified by
primers of both genes in a pair, the two genes were concluded
to be physically collocated. Because the BAC library has an
average insert size of 50 Kb [4], the two genes in each pair
were selected based on their distance in between smaller than
50 Kb. For each pair of genes, the primers for one of the
genes were used to amplify the entire BAC library of 43,000
clones [3] using a three-dimensional approach as described
by Ling and Chen [25]. To be more efficient, the primers
for the second gene in the pair were used to amplify only
the positive BAC clones from the screening. To speed up
the PCR screening, two pairs of primers for two genes with
similar annealing temperatures were used in a multiplex PCR
amplification.

Multiplex PCR was performed in a GeneAmp PCR Sys-
tem 9700 thermo-cycler. A 20 \( \mu \)L reaction mixture contained
1.0 \( \mu \)L (30 ng/\( \mu \)L) of a BAC clone DNA, 4.0 \( \mu \)L Mg-free 5X
PCR buffer (Promega, Madison, WI, USA), 0.1 \( \mu \)L of 5 unit
Tag DNA polymerase (Promega), 2 \( \mu \)L of 25 mM MgCl2,
0.5 \( \mu \)L of 2.5 mM dNTP (dATP, dCTP, dGTP and dTTP)

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Table 1: Primer sequences of Pst genes and amplification of same BAC clones. The table shows sequences and annealing temperatures (Tm) of primers based on Pst EST sequences used in PCR screening the Pst BAC library to determine the physical relationships of genes in pairs identified through BLAST search comparison with the Pgt whole genome sequence. The presence and absence of both genes of a pair in the same BAC clone are indicated by "Y" and "N", respectively.

| Gene pair | EST clone | Forward (5′−3′) | Reverse (5′−3′) | Tm (°C) | In same BAC clone |
|-----------|-----------|----------------|----------------|---------|------------------|
| 1         | PST78SP6L20C | CTGGTAATGGAGGTGGAACT | CTGGGGTAGGTAAGAAGGTC | 53.8/53.1 | Y               |
| 2         | PST78SP15C21F | AAAGAACCTTACGCAAACT | CACTCCACGGATGGAAT | 51.0/52.6 | Y               |
| 3         | PST78Ha6E1 | GACTTTATCGCATTTGCGAA | TGGACGAAGATCCCTTTGGA | 51.2/51.8 | N               |
| 4         | PST78SP19J9F | AGATACACGACCAAGAG | TATGATTCGTGGATGGAAC | 52.0/53.0 | Y               |
| 5         | PST78SP65T3C | CAGGAAGGAACAACACATTAA | ATGCAGTACAGACCCATCAA | 52.8/52.9 | Y               |
| 6         | PST78Ha10015 | GGACTACAGGCGACCACT | CACCCATACTGTCAAGG | 50.5/50.0 | Y               |
| 7         | PST78HaC375 | CATGCTGCTCTAATAATCTTCA | TCCCGACGACTTCTTTTAT | 52.6/54.1 | N               |
| 8         | PST78HaC443 | CAGAATAGACAAAGGC | ATGAGGACAGACCCATCAA | 50.7/51.2 | Y               |
| 9         | PST78SP6D14F | GAATCCAGACGCGCCAA | TACGCAGTACAGACCCATCAA | 53.7/56.4 | N               |
| 10        | PST78SP14N13F | AACAGACGCGCCAA | TGTACAGTACAGACCCATCAA | 50.8/50.1 | Y               |
| 11        | PST78HaC60 | GGGCTTGCGCGATGTTAAGGCA | CAGGAAGGAACAACACATTAA | 52.8/52.9 | Y               |
| 12        | PST78SP20d10F | CAGCCGGAGGGCAC | ATGAGGACAGACCCATCAA | 50.8/50.1 | Y               |
| 13        | PST78SP14M14 | TCAGAAGAACAAGCCACC | CCCATTTACCTGCACTTACAA | 55.0/55.0 | N               |
| 14        | PST78SP18H12F | CACCCGGAGGGCA | ATGAGGACAGACCCATCAA | 55.3/55.0 | N               |
| 15        | PST78SP2267 | GAGCTGGGTGTATCATGGCA | CGGGGACGACGCCAGAC | 58.5/58.5 | Y               |
| 16        | PST78SP220C | CAACTGCGAGCGGCA | CGGGGACGACGCCAGAC | 58.4/58.5 | N               |
| 17        | PST78SP66B11CF | ATGGACGCAGTCGA | CGGGGACGACGCCAGAC | 51.2/51.9 | Y               |
| 18        | PST78SP18M12F | CACCCGGAGGGCA | ATGAGGACAGACCCATCAA | 51.2/51.9 | Y               |
| 19        | PST78SP10f11F | CCGGTTGCGCGATGTTAAGGCA | CAGGAAGGAACAACACATTAA | 50.7/51.2 | Y               |
| 20        | PST78SP11F24F | CGGGGACGACGCCAGAC | ATGAGGACAGACCCATCAA | 50.7/51.2 | Y               |
| 21        | PST78SP12H1F | CAACTGCGAGCGGCA | CGGGGACGACGCCAGAC | 51.2/51.9 | Y               |

(Sigma Chemical Co., St. Louis, MO, USA), and 1.0 μL of 10 mM each primer synthesized by Operon Biotechnologies, Inc. (Huntsville, AL, USA). After 2 minutes of denaturation at 95°C, amplifications were programmed for 35 cycles, each consisting of 30 seconds at 95°C, 30 seconds at 59.4°C depending upon primer pairs shown in Table 1, 40 seconds at 72°C, and followed by a 10-minute extension step at 72°C. After PCR amplification, 5 μL of the solution for each sample was electrophoresed in a 1.5% agarose gel in 0.5x TBE buffer (0.089 M Tris-borate, 0.089 M boric acid and
3. Results

3.1. Homology of Pst ESTs and Pgt Genomic Sequences. Of the 4,219 Pst unisequences from the Ured, GermUred and Haus libraries were searched for homologous sequences in the Pgt genome, 1,432 had significant homology (e value < 1.00E-5) to Pgt genomic sequences. As shown in Table 2, the three libraries had different percentages of homologous genes with Pgt. The Ured library had the highest percentage, 54.51%, followed by the GermUred library (51.21%), while the Haus library had the lowest percentage (13.65%). In average, 33.94% of the 1,432 Pst genes had significant homology with the Pgt sequences.

| Pst cDNA library         | No. of unigenes | No. of unigenes with Significant Homology to Pgt genes | Percentage (%) of unigenes with significant Homology to Pgt genes |
|--------------------------|-----------------|-------------------------------------------------------|-----------------------------------------------------------------|
| Urediniospores           | 1,306           | 712                                                   | 54.51                                                          |
| Germinated urediniospores| 869             | 441                                                   | 51.21                                                          |
| Haustoria                | 2,044           | 279                                                   | 13.65                                                          |
| Total                    | 4,219           | 1,432                                                 | 33.94                                                          |

* The E value of 1 × 10⁻⁵ was used as a cut point to determine significant homology.

3.2. Physical Groups. The 1,432 Pst genes were aligned to 237 physical groups corresponding to 237 Pgt supercontigs (Supplementary file 1). As an example, Figure 1 shows Pst genes aligned to Pgt supercontig 1. The number of genes for each supercontig from each Pst cDNA library is shown in Table 3. The 237 physical groups ranged from 2,878 to 3,081,398 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)). Overall, the 1,432 genes matched 787,413 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)). Overall, the 1,432 genes matched 787,413 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)). Overall, the 1,432 genes matched 787,413 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)). Overall, the 1,432 genes matched 787,413 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)). Overall, the 1,432 genes matched 787,413 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)). Overall, the 1,432 genes matched 787,413 bp with most of the groups ranging from 5.0 Kb to 2.0 Mb (Figure 2(a)).

3.3. Exons of Pst Genes Revealed by Comparison with Pgt Genomic Sequences. Of the 1,432 Pst genes, 911 (63.62%) had more than one exon and the remaining 521 (36.38%) had one exon. Of the 911 genes with multiple exons, 570 (62.57%) had two, 200 (21.95%) had three, 97 (10.65%) had four, 25 (2.74%) had five, 13 (1.43%) had six, 3 (0.33%) had seven, 2 (0.22%) had eight, and 1 (0.11%) had nine exons. The different numbers of exons indicate the different levels of complexity of the genes, which might reflect their variability resulting from the evolutionary process.

3.4. Validation of Physical Relationships of Selected Pst Genes. To validate the physical relationships of Pst genes, a total of 84 forward and reverse primers were designed for 42 genes to form 21 pairs (Table 1). The genes in each pair were selected based on their proximity within 50 Kb in the physical map. Clones that were positively amplified with the first pair of primers resulted from the three-dimensional pooling screening were amplified with the second pair of primers, as illustrated in Figure 3. Of the 21 pairs of genes tested, 12 pairs (57%) were successfully identified in same BAC clones. The results clearly showed that these genes in pairs were truly colocalized in the Pst genome.

4. Discussion

Before the Pst genome is completely sequenced, which is under way, it is almost impossible to study genetic and physical relationships among genes of this obligate biotrophic
Table 3: Physical groups of *Pst* corresponding to *Pgt* supercontigs. Size of each *Pgt* supercontig and *Pst* EST coverage in the supercontig, number of contigs, number of ESTs in the urediniospore (Ured), germinated urediniospore (GermUred) and haustoria (Haus) libraries, and total average size of aligned ESTs and *Pst* gene density in each *Pst* physical group/*Pgt* supercontig.

| *Pst* physical group/*Pgt* supercontig | Size (bp) | No. of *Pst* ESTs covered | No. of *Pst* ESTs in each library and total | Average size (bp) of aligned *Pst* ESTs | *Pst* gene density in supercontig (bp/gene) |
|----------------------------------------|-----------|---------------------------|---------------------------------------------|---------------------------------------|---------------------------------------------|
| 1                                      | 3,081,398 | 29,571                    | 35 48 18 87 115                             | 193                                   | 20,140                                      |
| 2                                      | 2,570,998 | 18,205                    | 39 30 15 70 108                             | 158                                   | 22,357                                      |
| 3                                      | 2,616,274 | 16,097                    | 38 21 20 67 108                             | 149                                   | 24,225                                      |
| 4                                      | 1,978,325 | 9,744                     | 33 18 7 61 86                              | 113                                   | 23,004                                      |
| 5                                      | 2,008,477 | 15,513                    | 32 25 15 58 98                             | 158                                   | 20,495                                      |
| 6                                      | 1,808,965 | 14,784                    | 30 13 10 64 87                             | 170                                   | 20,793                                      |
| 7                                      | 1,797,936 | 8,626                     | 25 10 11 32 53                             | 163                                   | 33,923                                      |
| 8                                      | 1,737,638 | 10,749                    | 30 4 11 60 75                             | 143                                   | 23,169                                      |
| 9                                      | 1,714,174 | 14,977                    | 25 12 13 46 71                             | 211                                   | 24,143                                      |
| 10                                     | 1,640,743 | 14,619                    | 25 33 19 42 94                             | 156                                   | 17,455                                      |
| 11                                     | 1,547,344 | 12,529                    | 26 12 7 66 85                             | 147                                   | 18,204                                      |
| 12                                     | 1,543,397 | 8,518                     | 20 8 4 42 54                              | 158                                   | 28,581                                      |
| 13                                     | 1,556,540 | 8,681                     | 23 7 10 36 53                             | 164                                   | 29,369                                      |
| 14                                     | 1,510,324 | 12,100                    | 26 16 11 46 73                             | 166                                   | 20,689                                      |
| 15                                     | 1,374,611 | 15,098                    | 24 16 10 53 71                             | 213                                   | 19,361                                      |
| 16                                     | 1,217,956 | 12,940                    | 22 11 10 49 70                             | 185                                   | 17,399                                      |
| 17                                     | 1,242,959 | 18,124                    | 28 25 9 74 109                            | 166                                   | 11,403                                      |
| 18                                     | 1,195,459 | 5,965                     | 16 10 6 28 44                             | 136                                   | 27,170                                      |
| 19                                     | 1,137,327 | 7,582                     | 13 6 3 49 58                             | 131                                   | 19,609                                      |
| 20                                     | 1,198,131 | 9,437                     | 24 9 5 42 56                             | 169                                   | 21,395                                      |
| 21                                     | 1,084,580 | 7,679                     | 17 8 6 49 63                             | 122                                   | 17,216                                      |
| 22                                     | 1,051,806 | 5,445                     | 17 6 9 13 28                             | 194                                   | 37,565                                      |
| 23                                     | 1,003,138 | 10,246                    | 10 8 8 29 45                             | 228                                   | 22,292                                      |
| 24                                     | 1,008,357 | 9,373                     | 18 13 8 34 55                             | 170                                   | 18,334                                      |
| 25                                     | 1,068,291 | 9,855                     | 18 17 10 34 61                             | 162                                   | 17,513                                      |
| 26                                     | 1,006,249 | 10,628                    | 19 8 6 45 59                             | 180                                   | 17,055                                      |
| 27                                     | 1,005,714 | 8,119                     | 15 7 5 36 48                             | 169                                   | 20,952                                      |
| 28                                     | 964,966   | 6,986                     | 14 10 13 20 43                             | 162                                   | 22,441                                      |
| 29                                     | 999,150   | 4,693                     | 16 5 5 24 34                             | 138                                   | 29,387                                      |
| 30                                     | 919,905   | 7,064                     | 10 14 7 22 43                             | 164                                   | 21,393                                      |
| 31                                     | 986,084   | 6,076                     | 13 7 3 27 37                             | 164                                   | 26,651                                      |
| 32                                     | 894,979   | 7,030                     | 16 7 6 40 53                             | 133                                   | 16,886                                      |
| 33                                     | 889,308   | 108,494                   | 3 17 8 20 45                             | 2411                                  | 19,762                                      |
| 34                                     | 856,319   | 4,895                     | 8 5 4 22 31                             | 158                                   | 27,623                                      |
| 35                                     | 904,227   | 9,214                     | 20 16 3 33 52                             | 177                                   | 17,389                                      |
| 36                                     | 867,522   | 3,694                     | 10 5 3 11 19                             | 194                                   | 45,659                                      |
| 37                                     | 820,150   | 3,615                     | 12 5 0 22 27                             | 134                                   | 30,376                                      |
| 38                                     | 803,102   | 3,888                     | 9 8 8 7 23                             | 169                                   | 34,917                                      |
| 39                                     | 752,863   | 4,021                     | 11 3 4 28 35                             | 115                                   | 21,510                                      |
| 40                                     | 667,254   | 18,530                    | 11 6 8 24 38                             | 488                                   | 17,559                                      |
| 41                                     | 658,202   | 4,815                     | 8 11 5 5 21                             | 229                                   | 31,343                                      |
| 42                                     | 682,257   | 6,622                     | 10 10 4 26 40                             | 166                                   | 17,056                                      |
| 43                                     | 654,820   | 3,637                     | 12 7 4 20 31                             | 117                                   | 21,123                                      |
| 44                                     | 680,700   | 4,941                     | 13 2 1 33 36                             | 137                                   | 18,908                                      |
| 45                                     | 611,533   | 5,287                     | 9 8 2 30 40                             | 132                                   | 15,288                                      |
| 46                                     | 603,048   | 3,148                     | 10 8 5 9 22                             | 143                                   | 27,411                                      |
Table 3: Continued.

| Pst physical group/Pgt | Pst EST Contigs covered by Pst ESTs | No. of Pst ESTs in each library and total | Average size (bp) of aligned Pst ESTs | Pst gene density in supercontig (bp/gene) |
|------------------------|----------------------------------|------------------------------------------|--------------------------------------|----------------------------------------|
| Size (bp) in Pgt       | No. of ESTs in each library       | Ured GermUred Haus Total                 |                                       |                                        |
| 47 640,690             | 3,675                            | 12 9 6 10 25                            | 147 25,628                            |                                        |
| 48 569,981             | 2,235                            | 7 5 3 15 23                            | 97 24,782                             |                                        |
| 49 609,911             | 2,601                            | 9 2 3 16 21                            | 124 29,043                            |                                        |
| 50 685,923             | 5,158                            | 15 2 2 43 47                           | 110 14,594                            |                                        |
| 51 574,326             | 2,770                            | 9 8 4 14 26                            | 107 22,089                            |                                        |
| 52 572,077             | 2,928                            | 9 10 1 9 20                           | 146 28,604                            |                                        |
| 53 591,244             | 2,420                            | 9 2 1 15 18                           | 134 32,847                            |                                        |
| 54 525,265             | 6,453                            | 8 2 23 27                             | 239 19,454                            |                                        |
| 55 542,982             | 2,301                            | 7 7 3 6 16                            | 144 33,936                            |                                        |
| 56 577,102             | 1,706                            | 9 5 0 11 16                           | 107 36,069                            |                                        |
| 57 480,201             | 2,436                            | 5 2 3 16 21                           | 116 22,876                            |                                        |
| 58 496,650             | 2,974                            | 10 8 4 10 22                          | 135 22,575                            |                                        |
| 59 489,205             | 5,657                            | 5 4 3 13 20                           | 283 24,460                            |                                        |
| 60 436,003             | 2,794                            | 4 2 3 13 18                           | 155 24,222                            |                                        |
| 61 469,309             | 5,578                            | 11 6 6 20 32                          | 174 14,666                            |                                        |
| 62 428,160             | 8,885                            | 9 30 6 34 70                           | 127 6,117                             |                                        |
| 63 433,102             | 1,713                            | 7 6 2 7 15                           | 114 28,873                            |                                        |
| 64 440,512             | 1,559                            | 10 2 2 9 13                           | 120 33,886                            |                                        |
| 65 407,335             | 1,490                            | 5 1 2 7 10                           | 149 40,734                            |                                        |
| 66 388,993             | 1,775                            | 6 3 2 8 13                           | 137 29,923                            |                                        |
| 67 403,504             | 5,231                            | 9 4 2 22 28                           | 187 14,411                            |                                        |
| 68 403,089             | 2,587                            | 8 6 5 8 19                           | 136 21,215                            |                                        |
| 69 367,522             | 1,464                            | 5 6 0 2 8                           | 183 45,940                            |                                        |
| 70 386,059             | 1,545                            | 5 0 1 8 9                           | 172 42,895                            |                                        |
| 71 392,332             | 2,229                            | 9 0 1 19 20                           | 111 19,617                            |                                        |
| 72 398,881             | 3,281                            | 12 6 1 15 22                           | 149 18,131                            |                                        |
| 73 360,371             | 5,159                            | 8 0 1 30 31                           | 166 11,625                            |                                        |
| 74 351,149             | 2,082                            | 5 2 5 2 9                           | 231 39,017                            |                                        |
| 75 350,882             | 796                              | 7 2 1 7 10                           | 80 35,088                             |                                        |
| 76 341,344             | 2,159                            | 4 2 0 14 16                           | 135 21,334                            |                                        |
| 77 304,582             | 1,463                            | 4 0 2 11 13                           | 113 23,429                            |                                        |
| 78 296,996             | 2,909                            | 2 5 6 6 17                           | 171 17,470                            |                                        |
| 79 286,933             | 1,664                            | 3 0 1 9 10                           | 166 28,693                            |                                        |
| 80 312,600             | 2,919                            | 7 2 0 21 23                           | 127 13,591                            |                                        |
| 81 341,312             | 1,538                            | 9 4 2 8 13                           | 118 26,255                            |                                        |
| 82 375,963             | 1,130                            | 5 1 0 6 7                           | 161 53,709                            |                                        |
| 83 301,412             | 4,474                            | 9 6 2 20 28                           | 160 10,765                            |                                        |
| 84 306,211             | 2,780                            | 4 6 4 5 15                           | 185 20,414                            |                                        |
| 85 286,187             | 2,285                            | 5 6 5 2 13                           | 176 22,014                            |                                        |
| 86 268,723             | 3,915                            | 6 2 2 23 27                           | 145 9,953                             |                                        |
| 87 273,404             | 1,018                            | 3 0 0 12 12                           | 85 22,784                             |                                        |
| 88 271,488             | 2,241                            | 4 5 1 7 13                           | 172 20,884                            |                                        |
| 89 281,218             | 997                              | 4 6 1 1 8                           | 125 35,152                            |                                        |
| 90 282,829             | 2,792                            | 5 1 2 7 10                           | 279 28,283                            |                                        |
| 91 268,653             | 2,792                            | 5 4 4 3 11                           | 254 24,423                            |                                        |
| 92 249,303             | 1,055                            | 4 2 2 2 6                           | 176 41,551                            |                                        |
| 93 254,338             | 2,955                            | 8 2 2 20 24                           | 123 10,597                            |                                        |
| 94 224,654             | 1,369                            | 6 3 1 8 12                           | 114 18,721                            |                                        |
| Pst physical group/Pgt supercontig | Size (bp) covered by Pst ESTs | No. of Pst ESTs in each library and total Ured GermUred Haus Total | Average size (bp) of aligned Pst ESTs | Pst gene density in supercontig (bp/gene) |
|-----------------------------------|--------------------------------|-------------------------------------------------|-------------------------------------|------------------------------------------|
| 95                                | 268,031                        | 845                                            | 3                                   | 1                                        | 3                                    | 7                              | 121                             | 38,290                        |
| 96                                | 240,035                        | 984                                            | 5                                   | 2                                        | 2                                    | 5                              | 9                                | 109                            | 26,671                        |
| 97                                | 283,953                        | 370                                            | 3                                   | 2                                        | 0                                    | 1                              | 3                                | 123                            | 94,651                        |
| 98                                | 259,162                        | 1,865                                          | 4                                   | 1                                        | 3                                    | 6                              | 10                               | 187                            | 25,916                        |
| 99                                | 245,804                        | 345                                            | 4                                   | 0                                        | 0                                    | 5                              | 5                                | 69                             | 49,161                        |
| 100                               | 212,873                        | 1,355                                          | 4                                   | 4                                        | 1                                    | 2                              | 7                                | 194                            | 30,410                        |
| 101                               | 249,761                        | 566                                            | 4                                   | 2                                        | 0                                    | 4                              | 6                                | 94                             | 41,627                        |
| 102                               | 224,856                        | 1,583                                          | 4                                   | 3                                        | 2                                    | 5                              | 10                               | 158                            | 22,486                        |
| 103                               | 231,854                        | 1,007                                          | 5                                   | 5                                        | 0                                    | 4                              | 9                                | 112                            | 25,762                        |
| 104                               | 230,218                        | 1,733                                          | 4                                   | 1                                        | 3                                    | 4                              | 8                                | 217                            | 28,777                        |
| 105                               | 209,493                        | 158                                            | 1                                   | 1                                        | 0                                    | 0                              | 1                                | 158                            | 209,493                       |
| 106                               | 179,026                        | 857                                            | 2                                   | 0                                        | 0                                    | 9                              | 9                                | 95                             | 19,892                        |
| 107                               | 191,896                        | 1,271                                          | 5                                   | 3                                        | 1                                    | 4                              | 8                                | 159                            | 23,987                        |
| 108                               | 172,930                        | 455                                            | 2                                   | 0                                        | 0                                    | 3                              | 3                                | 152                            | 57,643                        |
| 109                               | 232,811                        | 1,142                                          | 6                                   | 3                                        | 1                                    | 5                              | 9                                | 127                            | 25,868                        |
| 110                               | 174,613                        | 802                                            | 4                                   | 0                                        | 1                                    | 5                              | 6                                | 134                            | 29,102                        |
| 111                               | 183,422                        | 2,657                                          | 5                                   | 0                                        | 0                                    | 20                             | 20                               | 133                            | 9,171                         |
| 112                               | 205,437                        | 810                                            | 2                                   | 1                                        | 0                                    | 3                              | 4                                | 203                            | 51,359                        |
| 113                               | 163,692                        | 624                                            | 4                                   | 0                                        | 1                                    | 5                              | 6                                | 104                            | 27,282                        |
| 114                               | 173,085                        | 754                                            | 3                                   | 0                                        | 2                                    | 5                              | 7                                | 108                            | 24,726                        |
| 115                               | 158,618                        | 1,640                                          | 4                                   | 1                                        | 3                                    | 5                              | 9                                | 182                            | 17,624                        |
| 116                               | 160,911                        | 427                                            | 1                                   | 1                                        | 1                                    | 0                              | 2                                | 214                            | 80,456                        |
| 117                               | 159,147                        | 691                                            | 3                                   | 4                                        | 0                                    | 1                              | 5                                | 138                            | 31,829                        |
| 118                               | 164,903                        | 2,902                                          | 7                                   | 4                                        | 5                                    | 7                              | 16                               | 181                            | 10,306                        |
| 119                               | 180,073                        | 674                                            | 3                                   | 0                                        | 0                                    | 5                              | 5                                | 135                            | 36,015                        |
| 120                               | 132,002                        | 219                                            | 1                                   | 0                                        | 0                                    | 1                              | 1                                | 219                            | 132,002                       |
| 121                               | 136,681                        | 318                                            | 2                                   | 0                                        | 0                                    | 4                              | 4                                | 80                             | 34,170                        |
| 122                               | 141,975                        | 1,011                                          | 5                                   | 1                                        | 0                                    | 8                              | 9                                | 112                            | 15,775                        |
| 123                               | 142,526                        | 697                                            | 3                                   | 1                                        | 0                                    | 5                              | 6                                | 116                            | 23,754                        |
| 124                               | 122,507                        | 109                                            | 2                                   | 0                                        | 0                                    | 2                              | 2                                | 55                             | 61,254                        |
| 125                               | 141,384                        | 74                                             | 1                                   | 1                                        | 0                                    | 0                              | 1                                | 74                             | 141,384                       |
| 126                               | 132,883                        | 396                                            | 4                                   | 1                                        | 0                                    | 5                              | 6                                | 66                             | 22,147                        |
| 127                               | 158,529                        | 334                                            | 2                                   | 0                                        | 0                                    | 4                              | 4                                | 84                             | 39,632                        |
| 128                               | 133,434                        | 518                                            | 2                                   | 1                                        | 1                                    | 1                              | 3                                | 173                            | 44,478                        |
| 129                               | 156,670                        | 102                                            | 1                                   | 0                                        | 0                                    | 1                              | 1                                | 102                            | 156,670                       |
| 130                               | 127,762                        | 653                                            | 2                                   | 0                                        | 0                                    | 3                              | 3                                | 218                            | 42,587                        |
| 131                               | 114,788                        | 871                                            | 3                                   | 1                                        | 1                                    | 5                              | 7                                | 124                            | 16,398                        |
| 132                               | 135,433                        | 436                                            | 4                                   | 2                                        | 0                                    | 2                              | 4                                | 109                            | 33,858                        |
| 133                               | 128,998                        | 576                                            | 3                                   | 0                                        | 1                                    | 3                              | 4                                | 144                            | 32,250                        |
| 135                               | 163,446                        | 463                                            | 2                                   | 1                                        | 0                                    | 2                              | 3                                | 154                            | 54,482                        |
| 137                               | 105,823                        | 42                                             | 1                                   | 0                                        | 0                                    | 1                              | 1                                | 42                             | 105,823                       |
| 138                               | 90,221                         | 617                                            | 2                                   | 2                                        | 2                                    | 0                              | 4                                | 154                            | 22,555                        |
| 139                               | 93,586                         | 451                                            | 3                                   | 0                                        | 1                                    | 6                              | 7                                | 64                             | 13,369                        |
| 140                               | 96,224                         | 620                                            | 1                                   | 1                                        | 0                                    | 0                              | 1                                | 620                            | 96,224                        |
| 141                               | 98,031                         | 1,362                                          | 4                                   | 0                                        | 0                                    | 14                             | 14                               | 97                             | 7,002                         |
| 142                               | 132,305                        | 323                                            | 2                                   | 0                                        | 0                                    | 4                              | 4                                | 81                             | 33,076                        |
| 143                               | 87,623                         | 181                                            | 1                                   | 0                                        | 0                                    | 1                              | 1                                | 181                            | 87,623                        |
| 144                               | 85,648                         | 237                                            | 1                                   | 0                                        | 0                                    | 2                              | 2                                | 119                            | 42,824                        |
Table 3: Continued.

| Pst physical group/Pgt supercontig | Size (bp) covered by Pst ESTs | No. of Pst ESTs in each library and total | Average size (bp) of aligned Pst ESTs | Pst gene density in supercontig (bp/gene) |
|-----------------------------------|------------------------------|------------------------------------------|--------------------------------------|------------------------------------------|
|                                   | Size (bp)                    | Ured | GermUred | Haus | Total |                                        |                                        |
| 145                               | 74,269                       | 118  | 2        | 2    | 2     | 59                                        | 37,135                                |
| 146                               | 66,839                       | 291  | 1        | 0    | 0     | 1                                         | 291                                    |
| 147                               | 66,397                       | 266  | 2        | 1    | 0     | 2                                         | 89                                     |
| 148                               | 64,884                       | 982  | 3        | 3    | 4     | 2                                         | 109                                    |
| 150                               | 63,036                       | 779  | 3        | 4    | 2     | 0                                         | 150                                    |
| 151                               | 78,814                       | 2,274 | 4     | 0    | 0     | 12                                        | 12                                     |
| 152                               | 68,096                       | 1,607 | 2     | 0    | 0     | 11                                        | 11                                     |
| 153                               | 58,505                       | 159  | 1        | 0    | 0     | 1                                         | 159                                    |
| 154                               | 59,544                       | 409  | 0        | 2    | 0     | 1                                         | 136                                    |
| 155                               | 57,757                       | 158  | 1        | 0    | 0     | 1                                         | 158                                    |
| 156                               | 63,803                       | 440  | 1        | 0    | 0     | 2                                         | 220                                    |
| 159                               | 48,833                       | 198  | 2        | 0    | 0     | 2                                         | 99                                     |
| 161                               | 70,147                       | 322  | 1        | 0    | 0     | 3                                         | 107                                    |
| 162                               | 96,527                       | 127  | 2        | 1    | 0     | 1                                         | 64                                     |
| 163                               | 42,923                       | 1,510 | 1     | 1    | 0     | 5                                         | 252                                    |
| 164                               | 56,756                       | 571  | 1        | 0    | 0     | 5                                         | 5                                      |
| 165                               | 46,219                       | 239  | 1        | 0    | 0     | 5                                         | 48                                     |
| 166                               | 62,331                       | 189  | 1        | 0    | 0     | 1                                         | 189                                    |
| 167                               | 55,078                       | 172  | 1        | 0    | 0     | 3                                         | 57                                     |
| 169                               | 56,434                       | 309  | 1        | 0    | 0     | 3                                         | 103                                    |
| 170                               | 41,057                       | 492  | 2        | 0    | 0     | 4                                         | 123                                    |
| 171                               | 47,090                       | 263  | 1        | 0    | 0     | 2                                         | 132                                    |
| 172                               | 51,493                       | 74   | 1        | 0    | 0     | 1                                         | 74                                     |
| 173                               | 32,945                       | 87   | 1        | 0    | 0     | 2                                         | 44                                     |
| 178                               | 50,831                       | 239  | 1        | 1    | 1     | 0                                         | 120                                    |
| 180                               | 26,426                       | 118  | 1        | 0    | 0     | 1                                         | 118                                    |
| 181                               | 25,288                       | 543  | 1        | 0    | 1     | 5                                         | 91                                     |
| 182                               | 25,555                       | 54   | 1        | 0    | 0     | 1                                         | 54                                     |
| 184                               | 25,182                       | 550  | 1        | 2    | 0     | 1                                         | 183                                    |
| 189                               | 22,821                       | 40   | 1        | 1    | 0     | 0                                         | 40                                     |
| 191                               | 21,049                       | 312  | 1        | 0    | 0     | 1                                         | 312                                    |
| 193                               | 20,340                       | 312  | 1        | 0    | 0     | 1                                         | 312                                    |
| 195                               | 20,131                       | 38   | 1        | 0    | 0     | 1                                         | 38                                     |
| 197                               | 19,986                       | 343  | 1        | 0    | 1     | 0                                         | 343                                    |
| 202                               | 18,478                       | 80   | 1        | 0    | 0     | 1                                         | 80                                     |
| 203                               | 18,097                       | 65   | 1        | 1    | 0     | 0                                         | 65                                     |
| 204                               | 19,139                       | 43   | 1        | 0    | 0     | 1                                         | 43                                     |
| 205                               | 19,822                       | 671  | 2        | 0    | 0     | 6                                         | 112                                    |
| 207                               | 17,451                       | 498  | 1        | 0    | 0     | 4                                         | 125                                    |
| 209                               | 16,612                       | 166  | 1        | 0    | 0     | 1                                         | 166                                    |
| 210                               | 16,297                       | 163  | 1        | 0    | 0     | 1                                         | 163                                    |
| 213                               | 15,902                       | 189  | 1        | 0    | 0     | 2                                         | 95                                     |
| 214                               | 16,066                       | 603  | 1        | 0    | 0     | 3                                         | 201                                    |
| 216                               | 15,718                       | 318  | 1        | 0    | 0     | 2                                         | 159                                    |
| 219                               | 15,090                       | 55   | 1        | 0    | 0     | 1                                         | 55                                     |
| 220                               | 14,899                       | 34   | 1        | 0    | 1     | 0                                         | 34                                     |
| 221                               | 14,629                       | 71   | 1        | 0    | 0     | 2                                         | 36                                     |
| 225                               | 13,856                       | 702  | 1        | 2    | 1     | 0                                         | 234                                    |

Comparative and Functional Genomics
Table 3: Continued.

| Pst physical | Size (bp) covered by Pst ESTs contigs | No. of Pst ESTs in each library and total | Average size (bp) of aligned Pst ESTs | Pst gene density in supercontig (bp/gene) |
|--------------|--------------------------------------|------------------------------------------|--------------------------------------|------------------------------------------|
| group/Pgt supercontig | Size (bp) in Pgt | Ured | GermUred | Haus | Total | Pst ESTs | |
|----------------------|----------------|-------|-----------|-----|-------|----------|-----|
| 227                  | 13,483         | 1     | 1         | 0   | 0     | 1        | 124 | 13,483 |
| 228                  | 13,460         | 1     | 0         | 0   | 3     | 3        | 163 | 4,487  |
| 236                  | 12,526         | 1     | 0         | 0   | 1     | 1        | 158 | 12,526 |
| 237                  | 12,336         | 1     | 0         | 0   | 8     | 8        | 220 | 1,542  |
| 238                  | 12,284         | 1     | 2         | 0   | 0     | 2        | 309 | 6,142  |
| 239                  | 15,052         | 1     | 0         | 0   | 0     | 1        | 80  | 15,052 |
| 241                  | 12,067         | 1     | 3         | 1   | 4     | 8        | 161 | 1,508  |
| 246                  | 11,389         | 1     | 0         | 1   | 0     | 1        | 538 | 11,389 |
| 249                  | 10,855         | 1     | 0         | 1   | 0     | 1        | 35  | 10,855 |
| 256                  | 10,451         | 1     | 0         | 0   | 3     | 3        | 182 | 3,484  |
| 262                  | 10,127         | 1     | 0         | 0   | 1     | 1        | 78  | 10,127 |
| 265                  | 10,029         | 1     | 0         | 0   | 4     | 4        | 133 | 2,507  |
| 269                  | 9,743          | 1     | 0         | 0   | 1     | 1        | 129 | 9,743  |
| 268                  | 9,738          | 1     | 0         | 1   | 2     | 3        | 72  | 3,246  |
| 270                  | 9,523          | 1     | 0         | 0   | 1     | 1        | 125 | 9,523  |
| 271                  | 9,385          | 1     | 0         | 0   | 2     | 2        | 51  | 4,693  |
| 273                  | 9,255          | 1     | 0         | 0   | 1     | 1        | 96  | 9,255  |
| 276                  | 8,976          | 1     | 0         | 1   | 0     | 1        | 139 | 8,976  |
| 278                  | 8,937          | 1     | 0         | 0   | 1     | 1        | 70  | 8,937  |
| 286                  | 8,437          | 1     | 0         | 0   | 1     | 1        | 50  | 8,437  |
| 287                  | 8,404          | 1     | 0         | 0   | 3     | 3        | 72  | 2,801  |
| 291                  | 8,194          | 1     | 0         | 0   | 1     | 1        | 79  | 8,194  |
| 292                  | 8,128          | 1     | 0         | 0   | 4     | 4        | 117 | 2,032  |
| 293                  | 8,101          | 1     | 0         | 0   | 5     | 5        | 109 | 1,620  |
| 295                  | 8,046          | 1     | 1         | 1   | 0     | 2        | 432 | 4,023  |
| 298                  | 7,881          | 1     | 0         | 1   | 0     | 1        | 91  | 7,881  |
| 299                  | 7,678          | 1     | 0         | 0   | 5     | 5        | 409 | 1,536  |
| 307                  | 7,428          | 1     | 0         | 0   | 1     | 1        | 53  | 7,428  |
| 311                  | 7,373          | 1     | 0         | 0   | 1     | 1        | 83  | 7,373  |
| 313                  | 7,328          | 1     | 0         | 0   | 0     | 1        | 154 | 7,328  |
| 325                  | 6,734          | 1     | 0         | 0   | 2     | 2        | 169 | 3,567  |
| 331                  | 6,471          | 1     | 3         | 1   | 0     | 4        | 93  | 1,618  |
| 340                  | 6,192          | 1     | 0         | 0   | 3     | 3        | 94  | 2,064  |
| 351                  | 5,919          | 1     | 1         | 0   | 0     | 1        | 167 | 5,919  |
| 359                  | 5,526          | 1     | 0         | 0   | 3     | 3        | 201 | 1,842  |
| 360                  | 5,501          | 1     | 0         | 0   | 1     | 1        | 281 | 5,501  |
| 363                  | 5,438          | 1     | 0         | 0   | 4     | 4        | 119 | 1,360  |
| 368                  | 5,333          | 1     | 0         | 0   | 2     | 2        | 188 | 2,667  |
| 371                  | 5,291          | 1     | 0         | 0   | 4     | 4        | 96  | 1,323  |
| 374                  | 5,126          | 1     | 0         | 0   | 3     | 3        | 144 | 1,709  |
| 375                  | 5,108          | 1     | 0         | 0   | 1     | 1        | 266 | 5,108  |
| 376                  | 5,100          | 1     | 0         | 0   | 5     | 5        | 61  | 1,020  |
| 377                  | 5,096          | 1     | 0         | 0   | 1     | 1        | 63  | 5,096  |
| 384                  | 4,789          | 1     | 0         | 0   | 1     | 1        | 30  | 4,789  |
| 386                  | 4,740          | 1     | 0         | 0   | 1     | 1        | 98  | 4,740  |
| 392                  | 2,878          | 1     | 76        | 12  | 62    | 150      | 265 | 19     |
| Total                | 86,550,604     | 787,413 | 1,447     | 1,088 | 572  | 2,952    | 4,604 | 35,082 | 18,799 |
Figure 1: An example of physical maps for *Pst* ESTs based on corresponding sequence positions of homologous genes of *Pgt*. All 242 physical groups are presented in Supplementary file 1. The distance in mega base (Mb) is shown on the left. The clones in a group indicated by a vertical line are in the same contig and the start and end positions of the sequence matching the positions in the contig are shown in the "( )" following the clone identification number. The number after the "( )" indicates the number of the gene with multiple positions in the *Pgt* genome. An asterisk indicates that the number of matching base pairs is smaller than 100. The clones underlined were used in PCR amplification of the *Pst* BAC library.
fungus without sexual reproduction [2]. In this study, we explored the possibility to use the whole genome sequence of 

*Pgt*, the most closely related fungus sequenced so far, as a reference to construct physical maps for 

*Pst* genes. From a total of 4,219 unique genes, we identified 1,432 genes significantly homologous to sequences in the *Pgt* genome. Because of their high nucleotide identities to the *Pgt* genome sequences, we assumed that these genes should have high levels of synteny to the corresponding genes in the *Pgt* genome. Thus, using the *Pgt* genomic sequences, we grouped

the 1,432 *Pst* unique genes with a total of 4,604 genomic loci into 237 physical groups corresponding to *Pgt* supercontigs. The proximity physical relationship was demonstrated for 12 pairs of genes using our *Pst* BAC library [3]. This study is the first to report the physical relationships for *Pst* genes and is the first to use the whole-genome sequence of a fungal species to study physical relationships of genes in a related species among the cereal rust fungi.

The homologous genes did not show an even distribution on the *Pgt* genome because no homologous *Pst* genes were found on 145 of a total of 382 *Pgt* contigs and gene densities varied greatly from 1,020 bp to 209,493 bp. Such an uneven distribution may be partially due to the different sizes of the *Pgt* supercontigs. The uneven distribution also could be caused by the relatively small number of genes. The 1,432 *Pst* genes are only about 8% of the total estimated number of genes based on the over 20,000 genes of *Pgt*. It also is possible that the genes expressed in each of the three developmental stages may cluster on certain genome regions. Nevertheless, the data may indicate the existence of gene-rich and gene-poor regions in the *Pst* and *Pgt* genomes. The information of gene-rich regions and *Pst*/*Pgt* homologous gene-rich regions will be useful in understanding the evolutionary relationships of the two related but different rust fungi. This hypothesis would be more clearly tested by comparing all *Pst* genes after the completion of the whole-genome sequencing and sequencing of more ESTs, which are currently being undertaken.
In this study, we only tested 42 Pst genes in 21 pairs in the PCR screening of the BAC library. In contrast to the 12 pairs that were demonstrated in the same BAC clones, positive results were not obtained for 9 of the 21 pairs. However, the unsuccessful amplification by the second genes in the 9 pairs does not exclude the possibility of physical relationships for the genes in each of these pairs. As the inserts of the BAC clones were relatively short, 50 Kb in average [3], the clones might be too small to harbor both genes in a pair. It is also possible that the Pst genes in each pair may have a longer distance than the reference distance in the Pgt genome, but they may still be linked to each other.

The Pst genes used in this study were from three libraries. The genes from the Ured library gave the highest percentage of genes homologous to Pgt and the genes from the Haus library gave the lowest percentage of homologous genes. The GermUred clones had similar percentage of Pgt-homologous genes to the Ured library, although the two libraries were made from different isolates while the Haus library was made with the same isolate as the Ured library [4–6]. The low proportion of the Pst genes from the Haus library similar to the Pgt sequences was surprising as we thought that two fungal species in the same genus should have higher homology than human and mouse that are in very different taxa [9]. Although this phenomenon needs more studies, we have learned from other rust fungi that genes expressed in haustoria tend to be more species specific [26, 27]. Comparisons of Pst genes expressed in different growth stages with the Pgt sequences tell us that genes expressed in urediniospore are more conserved among different Puccinia species while those expressed in haustoria are more unique. Such genetic differences may be related to their different requirements in temperature for infection of the same wheat host crop.

It is interesting that the smallest number of unique genes (279) from the Haus library produced the highest number (2,952) of genomic loci along the Pgt genome among the three libraries. The high fold (10.58x) of gene copies may compensate for the low number of homologous genes from haustoria, which may make the overall homology of Pst and Pgt genome sequences reasonably high. The genomic loci were aligned to more supercontigs than the genes from the Ured and GermUred libraries. These results indicate that haustorially expressed genes tend to have multiple copies and spread along the Puccinia genome. This phenomenon needs to be further studied using the whole genome sequence of Pst.

Although much of the physical relationship is still hypothetical and needs to be verified by the whole genome sequence of Pst, the physical groups constructed in this study can serve as references and starting points in assisting sequence assembling and gene annotation. A more detailed dissection of gene sequences, organization, structures, and clusters may allow us to pick genome regions and gene clusters to study their functions and developing molecular markers to tag virulence groups and characterize Pst populations.

In this study, we found that some ESTs could be matched to more than one location. Also, an alignment consisted of multiple exons while others do not have introns. We included the intronless sequences in the physical maps. Intronless sequences as pseudogenes have coincident nucleotide sequences with coding protein genes ubiquitously existing in the eukaryotes genome [28, 29]. Although pseudogenes may be functionless DNA fragments ubiquitously existing in the genome, they have evolved from mRNA reverse transcription and then reset in the genome. So, pseudogenes do not have introns and promoters but have poly(A) sequences. For a full-scale gene mapping, it represents the real gene transcription and sequence existence. Most of our EST sequences are not full-length and only have partial information of genes. This might be an explanation why a considerable number of ESTs were aligned to regions of the Pgt genome without introns.

We found that many of the Pst ESTs that matched to Pgt genomic sequences were shorter than 100 bp. These short sequences may be exons, whose lengths can vary greatly. Most vertebrate exons are between 50 and 400 bp long [30]. Using the complementary sequence feature method in humans, Arabidopsis, Cryptococcus, and Plasmodium, Saeyes et al. [31] reported that one-third of all exons were smaller than 100 bp. Guðlaugsdóttir et al. [32] reported significant variation in exon length for human and fission yeast ranging from 1 to thousands of base pairs. Because exon sizes can vary from a few base pairs to thousands of base pairs, we reserved even the segments smaller than 50 base pairs, which may have saved some unknown information in alignment and make the information available for the future Pst genome research. The number of exons in a gene may indicate its stability or variability, which may allow us to choose genes for studying various aspects of pathogen biology. Genes with only one exon may be chosen to study the genetic relationships at a higher taxonomic level, such as species and formae specialiae, and those with multiple exons may be used to study genetic differences among isolates within a forma specialis. Genes with multiple exons may be better candidates for studying traits like virulence and adaptation to different environments as these traits have more variations.

In this study, we produced preliminary physical maps for Pst genes. The 4,604 genomic loci of 1,432 genes were placed on the physical map account about 8% of potential genes, if we assume that Pst and Pgt have a similar number of genes. Because we used only unique genes, some genes belonging to large families could be located on multiple genome sites. In the future, this physical map will be verified and ultimately be improved by the complete set of the Pst genes and connected with nontranscribed sequences. The physical groups should provide insights into gene organization, identification of functionally related genes, positional cloning of full-length genes, information on exons and introns, and assist in sequence assembly and gene annotation for the Pst whole-genome sequencing.

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