GENETIC MAPPING, EFFECTIVENESS, AND TEMPERATURE SENSITIVITY OF THE *TRITICUM AESTIVUM* GENES *LrW* AND *LrW2* WHICH CONFER RESISTANCE TO *PUCCINIA TRITICINA*

BY

COLIN W. HIEBERT

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Abstract

Leaf rust (*Puccinia triticina* Eriks.) of wheat (*Triticum aestivum* L.) causes economically significant yield loss in Canada. Host genetic resistance to leaf rust is an effective means of disease control. However, as populations of *P. triticina* evolve virulence, new sources of resistance need to be found. When new leaf rust resistance genes (*Lr*) are discovered, genetic mapping will prevent gene redundancy, and facilitates gene stacking.

One objective of this study was to place potentially unique genes, *LrW* and *LrW2*, in the wheat genetic map using haploid deficiency mapping, and microsatellite markers linked to these genes. The other objective was to determine the degree of resistance of *LrW* and *LrW2* to a range of virulence phenotypes representative of Canadian *P. triticina* populations, and to determine the temperature sensitivity of *LrW* and *LrW2* incompatible reactions.

Haploid deficiency mapping located *LrW* on chromosome 5B, and *LrW2* may be on chromosome 1D. Mapping with an F_2 population showed that the *LrW* locus is 14.1 cM from the microsatellite marker gwm443, on the short arm of chromosome 5B. No linkage between microsatellite markers on chromosome 1D and *LrW2* was found. However, evidence from the haploid deficiency mapping and mapping with F_2's suggests that *LrW2* could be on the short arm of chromosome 1D. *LrW* is effective against *P. triticina* populations in Canada. *LrW2*, although not as effective as *LrW*, was resistant to all *P. triticina* isolates tested in this study. The infection types found with both genes increased in severity with incubation temperatures $\geq 21°C$.

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1. Introduction

Leaf rust (*Puccinia triticina* Eriks.) of wheat (*Triticum aestivum* L.) causes an average annual grain yield loss of 5-15% in the Canadian prairies (Samborski, 1985). Since Canada produces a lot of wheat relative to its population (DePauw and Hunt, 2001), the economic impact of leaf rust is significant. Although chemical control of leaf rust is possible, the most economical method of disease control is host genetic resistance (McIntosh *et al.* 1995). Many different leaf rust resistance (*Lr*) genes have been identified. McIntosh *et a.l.* (1995) catalogue 61 different *Lr* genes and alleles, although more have since been identified. However, as resistance genes are deployed in wheat cultivars the *P. triticina* populations evolve to overcome resistance, and new sources of resistance must be found (Dyck and Kerber, 1985).

As potentially new *Lr* genes are identified they should be genetically mapped in order to prevent gene redundancy. Typically *Lr* genes are assigned to chromosomes and to chromosome arms using monosomic analysis (Sears, 1953), and with telocentric mapping (The and McIntosh, 1975). Various molecular marker types have also been used to map *Lr* genes, such as random amplified polymorphic DNA (RAPD) (Schachermayr *et al.* 1994; Schachermayr *et al.* 1995), amplified fragment length polymorphism (AFLP) (Prins *et al.* 2001), and microsatellite markers (Raupp *et al.* 2001). In the case of procedures that produce complex electrophoretic banding patterns (RAPD and AFLP), linked DNA amplicons have been converted to sequence tagged sites (STS), which are more usable in breeding applications (Schachermayr *et al.*; 1994 Schachermayr *et al.* 1995; Prins *et al.* 2001). Mapping with microsatellite markers is

convenient because they are codominant, produce chromosome specific alleles, and are relatively polymorphic (Röder *et al.* 1998).

Molecular markers linked to *Lr* genes should allow for the selection of different gene combinations (Autrique *et al.* 1995). This will prove important in breeding programs as *Lr* genes deployed singly lose their effectiveness rapidly (McIntosh *et al.* 1995) and combinations of *Lr* genes should provide longer-term effectiveness (Dyck and Kerber, 1985).

Thomas *et al.* (2001) described a method for assigning genes to chromosomes in wheat. This technique uses haploid-derived aneuploids to identify the critical chromosome. The gene of interest is carried by the haploid (n = 3x = 21) female, and is pollinated by normal (2n = 6x = 42) plants that lack the gene of interest. The progeny from this type of cross can be euploid or aneuploid. While most progeny will carry the gene of interest, some aneuploid hybrids within the population of hybrids some aneuploids are missing the gene. Thomas *et al.* (2001) identified plants missing their genes of interest using allele specific sequence characterized amplified regions (SCAR). These individuals are missing the marker and the gene because the haploid parent failed to transmit part or all of the relevant chromosome. Chromosome deficiencies are determined using microsatellite markers previously mapped to each chromosome. This technique may be characterized as haploid deficiency mapping (Julian Thomas, personal communication).

In this study two undeployed *Lr* genes were examined. The first gene, *LrW* (temporary designation), was found in accession V336, which originated from an Iranian wheat cultivar (Dyck and Jedel, 1989). The second gene, *LrW2* (temporary designation),

was found in accession V860, originating from Armenia (Dyck, 1994). Both V336, and V860 were from the A.E. Watkins wheat collection. In the above studies, *LrW* conditioned resistance when inoculated with each of the nine leaf rust races tested, and *LrW2* was resistant to nine of ten races tested.

The objectives of this study were 1) to assign *LrW*, and *LrW2* to chromosomes using haploid deficiency mapping, 2) to find microsatellite markers that are linked to these genes, 3) to test the effectiveness of these genes against additional virulence phenotypes (races) of *P. triticina*, and 4) to test the effect that incubation temperature has on expression of resistance of *LrW* and *LrW2*.

2. Literature Review

2.1 Wheat

Wheat (*Triticum aestivum* L.) is allohexaploid with a genomic constitution of 2n = 6x = 42, AABBDD, where A, B, and D represent different genomes derived from three diploid progenitors. The A genome donor was found to be *T. monococcum* L. (2n = 14, AA) through cytological studies (Sax, 1922). The donor of the D genome was thought to be *Aegilops squarrosa* L. (McFadden and Sears, 1946; 2n = 14, DD). This was confirmed by Riley and Chapman (1960). The progenitor of the B genome remains unclear. As a result many theories arose (Kimber and Riley, 1963; Athwal and Kimber, 1972; Jauhar *et al.* 1991). Some studies, such as Kimber and Riley (1963), suggested *Ae. speltoides* (2n = 14, BB) as the donor of the B genome. Molecular evidence agreed with these findings (Daud and Gustafson, 1996). These three genomes have been shown to be

homoeologous (Sears, 1954). Chromosomes that are homoeologous display relatively high levels of structural similarity and homology, and have some ability to pair and presumably cross over in the absence of homologues, but do not have the same level of similarity as two homologous chromosomes. Jauhar *et al.* (1991) found that the A and D genomes have a higher degree of similarity compared to the B genome based on their ability to pair.

Globally wheat is important economically and as a food staple. Canada produces 5% of the world's wheat supply amounting to approximately 26 million tones annually. Most of this is produced in the prairie provinces (DePauw and Hunt, 2001). About 80% of Canadian wheat is exported. Therefore the wheat industry is an important part the economies of Alberta, Saskatchewan, and Manitoba.

2.2 Wheat Leaf Rust

Wheat leaf rust is caused by the basidiomycete *Puccinia triticina* Eriks. (Div. Amastigomycota, Class Basidiomycetes, Subclass Teliomycetidae, Order Uredinales, Family Pucciniaceae; Bold *et al.* 1987), which is an obligate parasite and macrocyclic. The alternate host of *P. triticina* is *Thalictrum speciosissimum* L. (Anikster *et al.* 1997). Wheat leaves are usually infected by urediospores, and rarely by aeciospores (Figure 1). The sexual cycle does not occur frequently in nature, and thus is not important role in epidemiology, or in the origin of new races (Samborski, 1985). A system of nomenclature using a set of 12 near-isogenic lines (NILs) was reported by Long and Kolmer (1989). Races, or virulence phenotypes, are defined by their differential virulence on these NILs carrying single leaf rust resistance (*Lr*) genes, and are assigned a

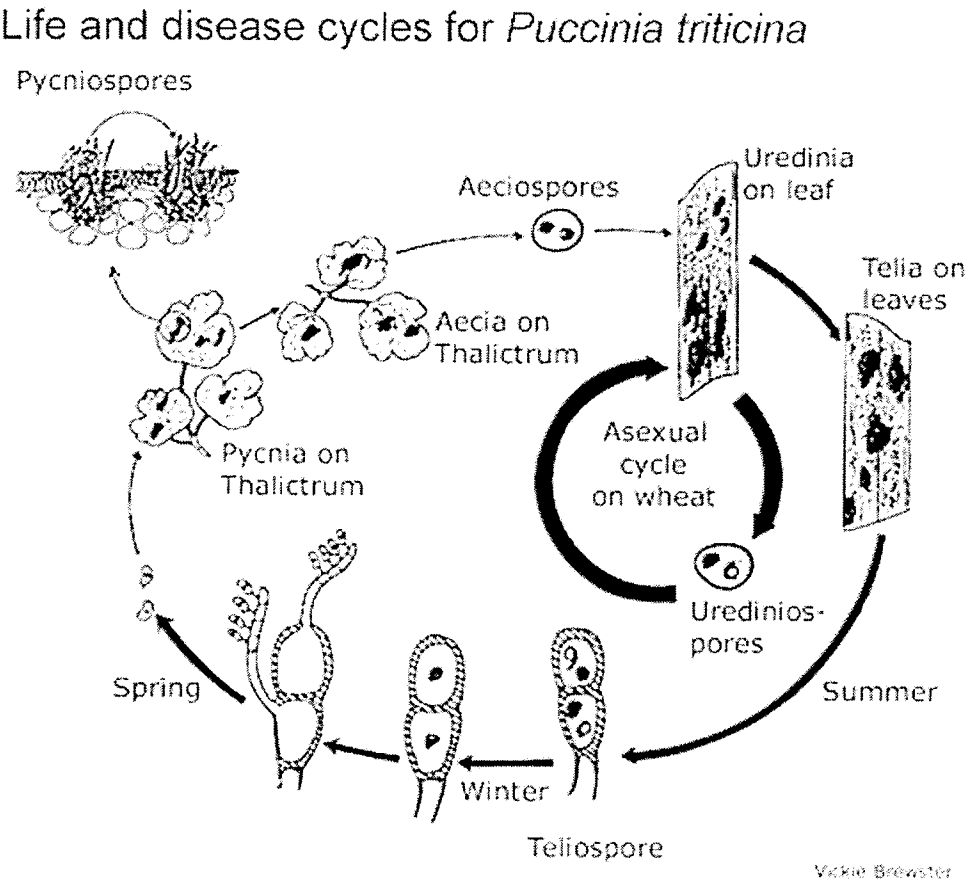

Figure 1 - Life cycle of *Puccina triticina*. Epidemics are caused by the polycyclic asexual production of urediniospores on wheat. The sexual cycle is of little significance in epidemiology in Canada. The disease cycle in Canada consists only of the asexual cycle. (Taken from http://www.cdl.umn.edu/gifs/prt-cycl.jpg)

three-letter code. Virulence phenotypes evolve by changes in their virulence on different *Lr* genes. This mostly occurs by mutation, plus asexual recombination may play a minor role (Samborski, 1985).

Urediospores can travel great distances. Canada receives the majority of its leaf rust inoculum from the United States (Roelfs, 1985). Leaf rust has a large economic impact. In the eastern prairies of Canada yield losses are frequently 5-15% if the cultivars grown have poor resistance, but losses can exceed that amount if environmental conditions permit (Samborski, 1985). In 1999 the Canadian prairies experienced one of the worst years in the past 20 years with yield losses of 5-20% (McCallum *et al.* 2000). This was caused by environmental conditions, large amounts of inoculum from the United States, and the leaf rust susceptibility of the most popular cultivar. In the most recent survey (2002), wheat leaf rust levels were quite high in 2002, but losses were not as high as 1999 (McCallum *et al.* 2003).

2.3 Host-Pathogen Interaction

The gene-for-gene theory of host-pathogen interactions was first demonstrated using flax (*Linum usitatissimum* L.) and flax rust (*Melamspora lini* Desm.) (Flor, 1956). The theory asserts that for avirulence genes in the pathogen there are corresponding resistance genes in the host. An incompatible interaction (failure to infect) occurs if any corresponding set of avirulence and resistance genes interact between the pathogen and host. The host is then said to be resistant. This interaction can be visualized using the quadratic check (Rowell *et al.* 1963; Figure 2A).

The gene-for-gene model more or less holds true for the wheat-leaf rust host-

A)

Resistance genes in the host	Avirulence genes in the pathogen: Av/-	av/av
R/-	-	+
r/r	+	+

B)

Resistance genes in the host	Avirulence genes in the pathogen: Av/Av	Av/av	av/av
R/R	-	+ or -	+
R/r	+ or -	+ or -	+
r/r	+	+	+

Figure 2 - Interaction of host resistance genes and pathogen avirulence genes. A) The classic quadratic check where resistance (R) and avirulence (Av) are dominant. B) The 3 x 3 table that accounts for the effects heterozygosity of R and Av genes might have on the host-pathogen interaction. A '+' interaction means there is a compatible interaction (host is susceptible), and a '-' interaction means there is an incompatible interaction.

pathogen system (Samborski and Dyck, 1968, 1976; Dyck and Samborski, 1970).However, the genetic states (homozygosity or heterozygosity) of resistance and avirulence genes affect the interaction in this system (Kolmer and Dyck, 1994). Infection type may vary with zygosity of the resistance and/or avirulence genes. Thus some of these genes displayed incomplete dominance. In fact, compatible reactions were seen in some cases where both the resistance gene and avirulence gene were putatively heterozygous. Kolmer and Dyck (1994) suggest a three-by-three table (Figure 2B) that accounts for the effects that heterozygosity can have on the host-pathogen interaction and is more complete than the quadratic check.

Host genetic resistance to *P. triticina* is subdivided into seedling resistance genes, which are effective from the seedling stage through maturity, and adult-plant resistance wherein the onset of resistance occurs after the seedling stage. Different types of resistance were reviewed by Dyck and Kerber (1985). Most *Lr* genes are of the race-specific, seedling variety. Several adult-plant *Lr* genes have shown race specificity. Only one gene seems to provide horizontal (race-nonspecific) resistance. That is the adult-plant gene *Lr34* (McIntosh *et al.* 1995).

Expression of resistance to leaf rust in wheat is affected by temperature. Dyck and Johnson (1983) found that infection types on *Lr* genes could decreases as temperature increases, increase as temperature increases, or remain the same across different temperatures. In a different study by Statler and Christianson (1993), where plants were grown in temperature ranges (cooler night temperatures and warmer day temperatures), it appeared that if *Lr* genes were temperature sensitive, low infection types could be achieved if the optimal temperature was within the temperature range that the plants were

grown. Temperature sensitivity also occurs for wheat genes conferring resistance to stem rust (Knott, 1981), caused by *Puccinia graminis* f. sp. *tritici* Eriks. And Henn., and to stripe rust (Park *et al.* 1992), caused by *P. striiformis* Westend. f. sp. *tritici* Eriks. Temperature sensitivity of rust resistance genes can also be affected by genetic state (homozygosity or heterozygosity) (Knott, 1981).

2.4 Mapping Leaf Rust Resistance Genes

Up to now, cytogenetic techniques have provided the principal means of gene mapping. Monosomics and telocentrics have been widely used in wheat to determine chromosome location and linkage to the centromere (McIntosh, 1987). Monosomics are individuals that have one copy of a given chromosomes rather than two. Telocentrics are chromosomes that have one arm missing. Since the telocentric is present in one or two doses the missing chromosome arm is either be hemizygous or completely deficient.

Monosomic analysis, as proposed by Sears (1953), involves crossing a line carrying the gene of interest with different monosomic lines and observing segregation in F_2 or F_3 populations. When a cross is made between the line carrying the gene and a monosomic that is deficient for the chromosome that the gene is located on, distorted, non-Mendelian segregation of resistance occurs in the progeny of monosomic F_1 plants. This distortion is due to the low frequency of nullisomics (no copies of a particular chromosome) that are recovered from self-pollinated monosomics. For example, Dyck *et al.* (1987) found that crosses of a resistant line with a putative new dominant leaf rust resistance (*Lr*) gene with all monosomics except 1B produced F_2 populations which fit the normal 3:1 (resistant : susceptible) single gene ratio. However, the cross involving mono 1B resulted

in a F_2 ratio that had significant overrepresentation of resistant progeny, and thus the 3:1 ratio did not fit. This means that this *Lr* gene, which was designated *Lr33*, was located on chromosome 1B. Many *Lr* genes have been located with this technique (e.g. *Lr9*, Sears, 1961; *Lr43*, Hussien *et al.* 1997). Dyck and Kerber (1981) used a modified version of this technique to locate *Lr30*, a recessive *Lr* gene. In this case the authors found expression of the recessive gene in the F_1 hybrids which would normally be masked by the alternate allele except when crossed with the critical monosomic when it is present in a hemizygous state.

Monosomic analysis may occasionally yield anomalous results. In an attempt to map an *Lr* gene, Singh *et al.* (2001) found that significant deviant segregation was found in F_3 families derived from crosses with both mono 2A and mono 5B. Further testing was needed to resolve which chromosome the gene was actually on. Sears (1961) appeared to find two disomic F_2 plants which otherwise were susceptible and one resistant F_2 that was nullisomic in a population that segregated as expected in a cross with the critical monosomic. With additional tests it was shown that contamination during pollination likely caused these anomalies to occur as the additional tests confirmed the tentative conclusions of monosomic analysis.

Telocentric chromosomes are useful for determining which chromosome arm a particular gene is on, and for detecting linkage between the gene and the centromere. A method for calculating both recombination and male transmission of the telocentric was developed by The and McIntosh (1975). This is done by crossing the line carrying the gene of interest with a monotelosomic (2n = 40 + telo), a monotelodisomic (2n = 41 + telo), or a ditelosomic (2n = 40 + 2 telos). The chromosome that is telocentric must be

the chromosome carrying the gene, and the arm present must be the arm carrying the gene, thus both the long and short arm telocentric must be studied. Then F_1 plants that are monotelodisomic are selected and allowed to self-pollinate. Chromosome number, including number of telosomes, and phenotype, such as rust reaction, are recorded in the F_2 allowing the application of the equations outlined by the authors. Several *Lr* genes have been mapped using this technique (Dyck and Kerber, 1981; Dyck *et al.* 1987).

Other variants of this technique have been used map *Lr* genes. *Lr32* was assigned to a chromosome using monosomic analysis (Kerber, 1987). To map the distance from *Lr32* to the centromere a line carrying *Lr32* was crossed with appropriate telosomic stocks of Chinese Spring (Kerber 1988). Hybrids that were monotelodisomic (2n = 41 + telo) were selected and used as males in a test-cross with Chinese Spring. If plants were disomic (2n = 42) and susceptible, or monotelodisomic (2n = 41 + telo) and resistant, they were classified as recombinants. Thus, recombination frequency could be directly calculated by dividing the number of recombinants by the total number of progeny.

With development of molecular markers, the location of wheat genes, including *Lr* genes, ca be mapped with increased precision. To determine linkage between molecular markers and a gene of interest, co-inheritance of the marker and the gene are studied in a segregating population (e.g. F_2; Raupp *et al.* 2001). Typically these *Lr* genes have been previously assigned to a chromosome. Where *Lr* genes are introgressed from wild relatives and are carried on translocated chromosomal segments, other strategies may be employed. For example markers can be found by studying near-isogenic lines (NIL) (Schachermayr *et al.* 1994; Schachermayr *et al.* 1995), or deletion stocks (Prins *et al.* 2001) carrying the *Lr* gene.

Lr genes have been marked using linked restriction fragment length polymorphism (RFLP) such as, *Lr19*, *Lr32* (Autrique *et al.* 1995), *Lr9* (Schachermayr *et al.* 1994; Autrique *et al.* 1995), and *Lr24* (Autrique *et al.* 1995; Schachermayr *et al.* 1995). These markers are based on the hybridization of radioisotope labeled DNA probes to genomic DNA that has been digested with restriction endonucleases. This process is laborious, and may yields low marker polymorphism (Röder *et al.* 1998). Markers that are highly specific for the gene of interest, and utilize the polymerase chain reaction (PCR) are more suitable for use in marker assisted breeding programs (Schachermayr *et al.*; 1994; Schachermayr *et al.* 1997; Huang and Gill, 2001; Prins *et al.* 2001).

PCR-based markers used to map *Lr* genes include random amplified polymorphic DNA (RAPD) (Schachermayr *et al.* 1994; Schachermayr *et al.* 1995), amplified fragment length polymorphism (AFLP) (Prins *et al.* 2001), and microsatellite markers (Raupp *et al.* 2001). Both RAPD (Schachermayr *et al.* 1994; Schachermayr *et al.* 1995) and AFLP (Prins *et al.* 2001) markers tightly linked to *Lr* genes have been converted to sequence-tagged-site (STS) markers. These are PCR-based markers that produce simple electrophoretic banding patterns, and allow high throughput (Schachermayr *et al.* 1997). RFLP markers tightly linked to *Lr* genes may be converted to STS markers (Schachermayr *et al.* 1997; Huang and Gill, 2001).

Lr genes deployed singly are expected to lose their effectiveness more rapidly than stacked combinations of genes (Dyck and Kerber, 1985; McIntosh *et al.* 1995). Reliable markers can allow specific *Lr* gene combinations to be selected efficiently (Schachermayr *et al.* 1994; Schachermayr *et al.* 1995; Hussien *et al.* 1997; Huang and Gill, 2001).

Accurate mapping of *Lr* genes, and proper genetic studies such as allelism tests, prevents confusion involved with gene names and identity as outlined in Huang and Gill (2001). Three genes introgressed into wheat from *Aegilops squarrosa* L., *Lr21*, *Lr39*, and *Lr40*, are potentially the same gene. The authors found that one source of *Lr39* was actually carrying the gene known as *Lr40*. Furthermore, *Lr40* was tightly linked in repulsion to *Lr21*, and the same STS marker detects both genes strongly suggesting allelism. Careful genetic studies allow the prevention of such situations. For example, Singh *et al.* (2001) found an unknown *Lr* gene in an Australian cultivar that was located on the same chromosome as a previously identified gene. Further testing showed that these genes were allelic. Contrasting reactions to different virulence phenotypes of *P. triticina* showed that these were different alleles at the same locus and not two sources of the same gene. Thus, gene mapping, allelism, and virulence tests are all required to prevent gene redundancy.

2.5 Use of Haploid Derived Aneuploids for Assigning Genes to Chromosomes

Thomas *et al.* (2001) describe a method of assigning genes to chromosomes as an alternative to monosomic analysis. The method involves generating an array of random aneuploid hybrids by pollinating haploid wheat (n=21) with euploid wheat (2n=42). Aneuploidy arises from abnormal meiosis of the haploid resulting in irregular gamete constitution. This was first observed for wheat by Sears (1939). In the method of Thomas *et al.* (2001) the haploid carries the gene of interest. While most hybrids will carry the gene, those that test deficient for the gene lack all or part of the critical chromosome from the haploid parent. The critical chromosome is then identified using

chromosome specific microsatellite markers. In the case of incomplete chromosome deficiencies the authors propose that homoeologous recombination may be occurring. The hybrids recovered range from disomics, to simple monosomics, to complex aneuploids. In this first study deficiency for the critical chromosome was identified using a linked SCAR marker rather than by detecting the gene's presence by observing phenotypes.

2.6 Haploid Wheat

Haploid wheat (n = 3x = 21, ABD), also referred to as a polyhaploid, can be generated in different ways. In some early research, wheat haploids were produced inadvertently as a result of random parthenogenesis (Gaines and Aase, 1926; Person, 1955). Intergeneric crosses have also produced haploids, although the objective of these experiments was to study intergeneric hybrids, not to generate haploid wheat (Sears, 1939; Riley and Chapman, 1957). Modern techniques for producing haploids include chromosome elimination, anther culture, and microspore culture.

Intergeneric crosses with wheat, where wheat is the female, generates wheat haploids by chromosome elimination (Barclay, 1975; Laurie and Bennett, 1988). Intergeneric pollination results in a fertilized embryo with a haploid chromosome set from each parent. Chromosomes are eliminated, for example in wheat by maize crosses, because of abnormal mitotic behavior of paternal chromosomes. Normal maize chromosome constrictions at the centromere and secondary constrictions are less visible or absent. These centromeres fail to function normally, have a low affinity for wheat spindle, and tend to lie away from the metaphase plate. Consequently the number of

maize chromosomes per cell decreases with each cell division until none remain. Most wheat embryos have lost all maize chromosomes after the third cycle of cell division (Laurie and Bennett, 1989). Embryo rescue is required to recover high frequencies of haploids due to early embryo and endosperm abortion (Laurie and Bennett, 1988, 1989). The number of embryos produced by wheat by maize crosses can be increased by treating wheat florets with synthetic hormones such as 2,4-dichlorophenoxyacetic acid (2,4-D). This results in an increased number of pollen tubes that reach the micropyle (Wedzony and Lammeren, 1996).

Many different pollinators have been used to produce wheat haploids by chromosome elimination including *Hordeum bulbosum* L. (Barclay, 1975), *Zea mays* L. (Laurie and Bennett, 1988), *Tripsacum dactyloides* (Li *et al.* 1996), and *Secale cereale* L. (Sears, 1939). Tetraploid *H. bulbosum* was thought to be a promising pollinator for haploid wheat production (Barclay, 1975). However two genes that restrict crossability between wheat and rye, *Kr1* (Riley and Chapman, 1967) and *Kr2* (Sitch *et al.* 1985), have been shown to cause incompatibility between genotypes of wheat and *H. bulbosum* (Snape *et al.* 1979; Falk and Kasha, 1981). In addition, some genetic control in *H. bulbosum* also impacts crossability, but this is mostly due to ploidy level (Sitch and Snape, 1986). Diploid *H. bulbosum* is less a less effective pollinator of wheat than tetraploid *H. bulbosum* (Barclay, 1975; Sitch and Snape, 1986). It was shown in durum wheat that germination of *H. bulbosum* pollen was constant across durum genotypes, however inhibition of pollen tube growth occurred in the ovaries (O'Donoughue and Bennett, 1994).

Producing haploid wheat by crossing with maize is advantageous because maize is relatively insensitive to *Kr1* and *Kr2* (Laurie and Bennett, 1987, 1988). Thus, the success rate of haploid wheat production is more consistent across wheat genotypes when pollinating with maize as compared to *H. bulbosum* (O'Donoughue and Bennett, 1994). However genotypes of wheat and maize do result in some variation in success rate. Genotype of durum wheat influences ovary development, embryo and plant formation, opposed to maize, where genotype only influences embryo formation (Cherkaoui *et al.* 2000). Similarly, in wheat (hexaploid) maize genotype influenced embryo formation (Verma *et al.* 1999).

Crossing wheat with *Tripsacum dactyloides* results in a higher rate of embryo formation compared to crossing with maize, and displays similar insensitivity to *Kr* genes. However, the haploid wheat plants recovered from such crosses frequently have *T. dactyloides* chromosomes that were not eliminated (Li *et al.* 1996).

Two methods of androgenesis can be used to recover haploid plants, anther culture and microspore culture. Anther culture is a process where microspores become embryogenic while in the anther. Uninucleate microspores, that are starch-free, develop into embryo-like structures from calli that progress through the normal embryo stages including the globular, heart, and torpedo stages (Nitsch and Nitsch, 1969). A drawback of anther culture is that many plantlets recovered are albino. The ability of wheat genotypes to generate green plants is under the additive polygenic control of nuclear genes (Zhou and Konzak, 1992). This could be partially overcome by increasing the number of plantlets produced, which is made possible by using modified culture media (Zhou and Konzak, 1989). Similar to male certation in monosomic wheat, gametic

selection favoring 21 chromosome microspores occurs in anther culture (DeBuyser *et al.* 1989).

Microspore culture is similar to anther culture in that microspores are become embryonic at the uninucleate stage. However unlike anther culture, microspore culture isolates individual microspores that produce pseudoembryos from microspores with fibrillar cytoplasm and avoid callus formation. This technique has been refined to the point where up to 5500 green plants can be produced from a single wheat spike (Liu *et al.* 2002). Microspore culture is advantageous because of the nearly 4-fold increase in green plant recovery compared to anther culture (Holme *et al.* 1999).

The time needed to produce haploid wheat by chromosome elimination or by androgenesis is approximately the same. However androgenesis is more cost effective on a per plant basis (Snape *et al.* 1986). Comparisons of genetic maps developed from doubled haploids arising from chromosome elimination (female meiosis) and anther culture (male meiosis) revealed significant differences in genetic distances between RFLP markers used to generate the map (Wang *et al.* 1995).

2.7 Behavior of Chromosomes in Haploids

Meiosis in haploids of a allopolyploid like wheat can be studied by directly observing meiotic cells in wheat haploids or chromosome behavior can be deduced by observing meiotic cells in interspecific and intergeneric hybrids between wheat and related species/genera. In these crosses no homologues are present but the genomes in the hybrids are homoeologous.

Synapsis is an essential process in normal meiosis, between zygotene and pachytene, which occurs between homologous chromosomes in euploid organisms. In haploid barely (*Hordeum vulgare* L., n = 7) complete intrachromosomal and interchromosomal synapsis occurs so that all or most chromosomal segments are paired despite the absence of homologues (Sadasiviah and Kasha, 1971). Similarly, in hybrids of *T. aestivum* (2n = 6x = 42, AABBDD) and *T. kotschyi* (2n = 4x = 28, $C^UC^US^VS^V$) near complete synapsis occurs in pachytene. Again, no homologues are present, however the five genomes present in the hybrid are homoeologous. Branched synaptonemal complexes were found in chromosomes involved in interchromosomal and intrachromosomal pairing. The presence or absence of the *Ph1* allele, which normally restricts homoeologous pairing, does not affect pairing in pachytene (Gillies, 1987). It appears that, in the absence of homologoues, lack of homology does not prevent chromosomes to synapse. Pairing can persist through diplotene, however the appearance of chiasmata and the number bivalents in diakinesis and metaphase I show sudden decline in associated chromosomes. Unassociated chromosomes become univalents. Univalents are distributed near the poles of the cell during diakinesis (Wagenaar, 1961a).

In metaphase I of haploids and intergeneric/interspecific hybrids there are two types of chromosomes that are of interest, those that are paired (mostly bivalents as trivalents and other multivalents occur at very low frequencies) and those that are unpaired (univalents).

Although most chromosomes in cells without homologues undergo synapsis, most of these prophase pairing relationships disappear in metaphase (Sadasiviah and Kasha, 1971; Gillies, 1987). In barley haploids a single bivalent (open) was only found in about

4% of metaphase I cells (Sadasiviah and Kasha, 1971). Haploids of wheat usually have one bivalent (usually open) per cell (Person, 1955; Riley and Chapman, 1957; Kimber and Riely, 1963; Jauhar *et al.* 1991). Pairing in haploid wheat occurs predominantly between homoeologous chromosomes, or at minimum is intergenomic (Jauhar *et al.* 1991), explaining why the number of bivalents found in a metaphase I cell increases with the number of homoeologous genomes present (Riley and Chapman, 1957). These bivalents disjoin in a normal fashion (Person, 1955), and result in duplications and deficiencies in cells where nuclei form following first division (Sears, 1939).

Although the formation of bivalents is interesting in terms of revealing homoeologous relationships and in their causation of duplications and deficiencies in potentially viable gametes in haploids, the behavior of univalents is more significant in generating viable gametes because most meiotic chromosomes (average of 19 of 21) in wheat haploids are univalents.

Univalents can be subdivided into two classes, those involved in secondary associations, and those that are completely independent from other univalents. There are two types of secondary associations that have been observed in meiosis of haploids, side-by-side associations, where chromosomes are aligned beside each other but do not have chiasmata, and end-to-end associations, in which chromosomes are associated at their ends in a chain-like fashion (Person, 1955; Riley and Chapman, 1957). End-to-end associations are believed to show no indication of homology, opposed to side-by-side associations, which are believed to occur between chromosomes that have significant homology, such as chromosomes from a homoeologous group, that have failed to form chiasmata. The fact that the number of bivalents per wheat haploid cell is inversely

proportional to the number of side-by-side associations appears to support this hypothesis (Person, 1955). A simplified view of how disjunction of side-by-side associations takes place is that they disjoin at the same time as bivalents (Person, 1955). If this were the case then migration of univalents to the cells poles would not be random. However, distribution of univalents has been shown to be random. Thus, side-by-side associations must disjoin in several ways, 1) univalents in side-by-side associations could disjoin before bivalents, therefore univalents could return to the metaphase plate and distribute randomly, 2) disjunction of side-by-side associations could coincide with bivalent disjunction, therefore producing the same affect of bivalents in haploids, or 3) side-by-side associations could fail to disjoin resulting in both univalents migrating to the same pole (Riley and Chapman, 1957). Distribution of univalents with no secondary associations is random (Person, 1955; Riley and Chapman, 1957).

Univalent accumulation at the metaphase plate (metaphase I) initiates anaphase I when a threshold number of univalents have moved to the metaphase plate from their initial polar distribution in diakinesis (Wagenaar, 1961a, b). The fate of univalents during the onset of anaphase I is dependent on their position relative to the metaphase plate. Univalents that have not reached the metaphase plate are pulled back to the nearest pole by the spindle apparatus. However, univalents that are found along the metaphase plate usually are oriented and sister chromatids separate (univalent disjunction) after bivalent disjunction (Wagenaar, 1961a). Cells with fewer bivalents, thus more univalents, usually take longer to accumulate enough univalents for the onset of anaphase I to occur, while cells with several bivalents proceed to anaphase more quickly (Wagenaar, 1961b).

Furthermore, when the rate of accumulation is slow more laggards are present during anaphase I (Wagenaar, 1961a).

Using hybrids of wheat and rye (no homologues present), C-banding of early anaphase I cells revealed recombination occurred between non-homologous chromosomes that were involved in intergenomic bivalents (Naranjo *et al.* 1989; Naranjo and Fernández, 1996). This demonstrates that intergenomic bivalents found in wheat and durum haploids (Jauhar *et al.* 1991; Jauhar *et al.* 1999) are likely to have crossed over.

If second division fails after bivalent disjunction and univalent distribution it is possible that haploids may produce viable gametes, which would often be aneuploid, if their chromosome constitution is not too irregular (Sears, 1939). This requires most univalents to orient and disjoin otherwise too many chromosome deficiencies will be present for viable gamete production.

2.8 Progeny and Gamete Formation of Haploids

Hybrids have been recovered when haploid wheat was pollinated by normal wheat (Sears, 1939; Riley and Chapman, 1957). The study by Sears (1939) examined the chromosome number and pairing of such hybrids. Of 13 hybrid plants recovered two had 42 chromosomes and always had 21 bivalents. Five plants had a somatic chromosome count of 41 and pairing of 21II + 1I, meaning these plants were monosomic for one chromosome. One plant was a double monosomic (40 chromosomes, 19II + 2I). The remaining plants contained different combinations of univalents, trivalents, and quadrivalents. Trivalents and quadrivalents were likely the products of non-homologous exchange (Sears, 1939).

Recovery of hybrids from pollinated haploids means that some female fertility exists in haploid wheat despite complete male sterility. Sears (1939) proposes four different models for how viable aneuploid gametes could be formed through meiosis in haploids. In the first model mostly univalents and a few, usually one, bivalent are oriented along the metaphase plate (Figure 3A). In first division univalents disjoin, that is sister chromatids separate, and bivalents disjoin normally. If second division fails two gametes are formed and duplications and deficiencies will exist for the chromosomes involved in bivalents. In the second model only univalents exist in metaphase I (Figure 3B). Most univalents are oriented on the metaphase plate, and disjoin as in the first model. However, any univalents not found along the metaphase plate both sister chromatids in the univalent will be in the same nucleus. If second division fails, viable gametes may be produced. In the simplest case the result would be an n+1 gamete and an n-1 gamete. This would produce simple monosomics and trisomics when pollinated by a euploid plant. The third model proposed by Sears involves restitution nucleus formation in first division (Figure 3C). However, if a univalent disjoins during first division both chromatids may be distributed in the same daughter nucleus after the remaining univalents disjoin in second division. This would result in one gamete with a chromosome deficiency and one gamete with a chromosome duplication. The fourth model also involves nuclear restitution in first division. However, if one or more

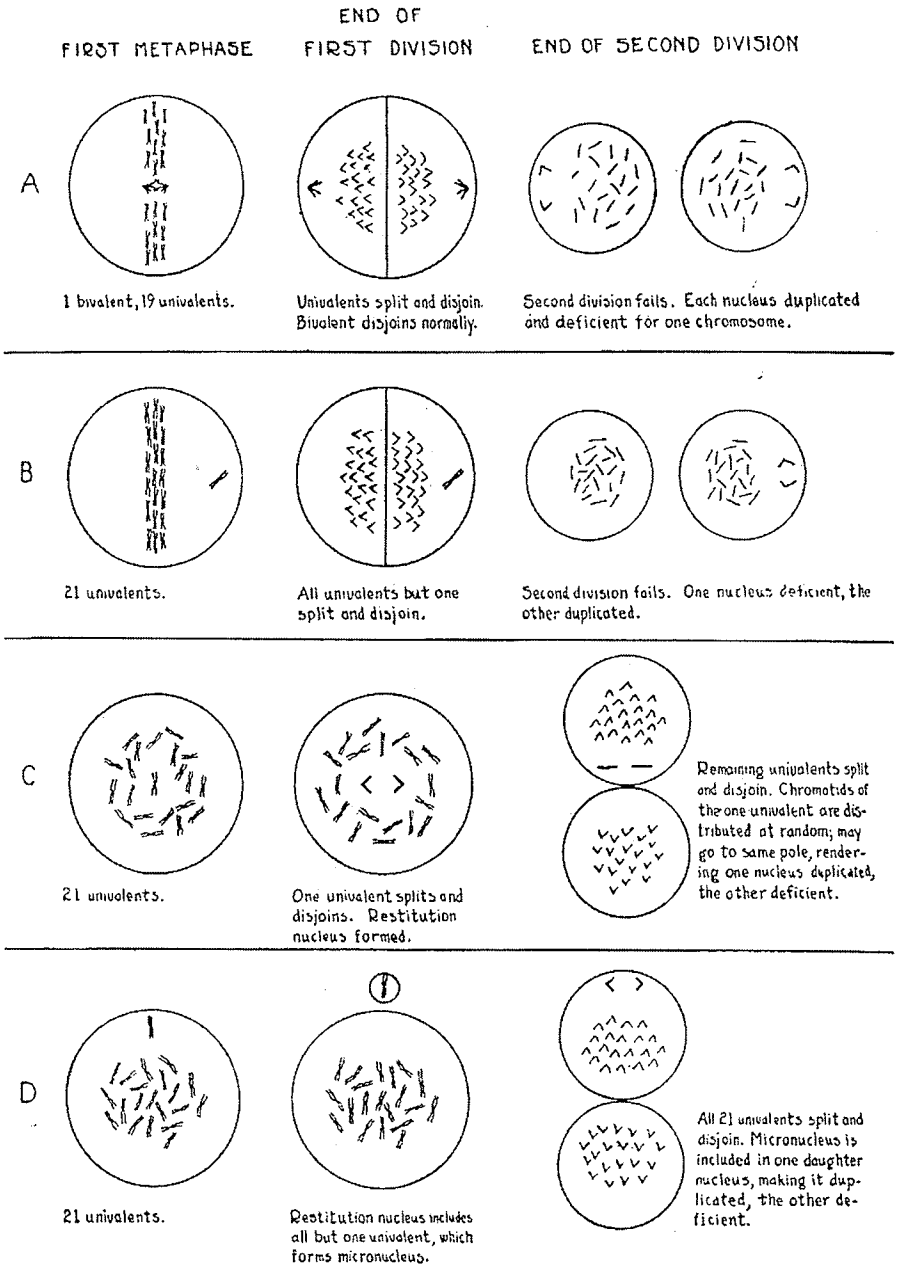

Figure 3 - Four models (A, B, C, and D) of viable gamete formation with chromosome number aberrations in wheat haploids (Sears, 1939).

univalents are involved in the formation of an adjacent micronucleus both chromatids of these univalents could be incorporated into one of the daughter nuclei (Figure 3D), or these chromatids could be lost. This could lead to two gametes that have chromosome deficiencies, or one gamete with deficiencies and one with duplications. Model one can explain both the appearance of univalents, and trivalents and quadrivalents in the progeny because of the potential for translocations exist. Sears (1936) points out that if recombination occurs and a single restitution nucleus forms at first division as seen by Gaines and Aase (1926) the occurrence of a reciprocal translocation in a gamete is possible.

Reciprocal translocations, trisomy, and monosomy were found in the progeny of haploids in *Sorghum* (Endrizzi and Morgan, 1955). Separation of sister chromatids in anaphase I was observed in *Sorghum* haploids (Reddi, 1968), and in wheat (Person, 1955; Wagenaar, 1961a). Evidence of sister chromatid disjunction in the meiosis of haploids supports the models of viable aneuploid gamete formation outlined by Sears (1939).

Viable female gametes in haploid wheat often have chromosome deficiencies (Sears, 1939). When chromosome deficiencies occur some loci are not transmitted from the haploid parent. Failure of chromosome transmission can be a tool for assigning genes to chromosomes (Thomas *et al.* 2001).

3. Materials and Methods

Generating haploids

Haploids were generated from wheat (*Triticum aestivum* L.) lines Tc-*LrW* (Thatcher*6/V336, RL6107; Dyck and Jedel, 1989), and Tc-*LrW2* (Thatcher*3/V860; Dyck, 1994). Plants were grown in growth cabinets at 18°C with the lights on (16h) and 16°C while dark (8h) to promote tillering. Haploid wheat (n = 3x = 21, ABD) plants were produced based of the procedure reported by Laurie and O'Donoughue (1994). Florets were emasculated the day before the anticipated onset of anthesis as determined by anther colour. Emasculated spikes were pollinated with maize (*Zea mays* L.) one day after emasculation. Then spikes were treated with dicamba (100 ppm) at one, and two days after pollination, misting with an atomizer to the point of runoff. Sixteen days after pollination caryopses were removed from the plant, and embryos were excised in a sterile environment. Embryos were placed in 25ml screw cap glass vials containing media (11g agar, 40g sucrose, and 2.75g Gamborg's B5 in 1 litre). The embryos were subjected to 4 days of cold treatment at 4°C in the dark, 3 days of dark treatment at room temperature (approximately 20°C), and then were placed under lights (14 hrs light, 10 hrs dark) at 20°C. Once roots, and a shoot of approximately 2-3 cm developed the plantlets were transplanted into three-inch pots containing soilless mix and grown in a growth cabinet with 16 hours of light at 17°C, and 8 hours dark at 16°C. When plants were at the three-leaf stage they were transplanted into larger pots with a soil mix.

Pollinating haploids

Twenty haploid plants of Tc-*LrW*, and twenty haploids of Tc-*LrW2*, were grown in a growth cabinet at 18°C with the lights on (16 hr), and 16°C when dark (8 hr). Florets on the haploid plants were pollinated with pollen from plants of cv. AC Foremost (HY320*5/BW533//HY320*6/7424-BW5B4). AC Foremost is susceptible to many isolates of leaf rust including virulence phenotype MBDS (P. Seto-Goh, personal communication). Pollinators were prepared by clippng detached heads of AC Foremost in anthesis thereby stimulating further florets of open and shed pollen. Haploid heads were clipped but not emasculated and pollinated 2 to 4 heads at a time in a glassine bag. The florets of haploid wheat do not need to be emasculated because they are completely male sterile but can produce some hybrid seed when pollinated with pollen from euploid wheat because of limited female fertility (Sears, 1939). Hybrid seed was harvested at maturity.

Testing for leaf rust susceptible hybrids

Hybrids derived from haploids were inoculated with *P. triticina* virulence phenotype MBDS (Long and Kolmer, 1989; McCallum and Seto-Goh, 2003) by suspending urediospores in light oil (Bayol 55, Imperial Oil Canada, Toronto, ON), and spraying the oil/spore mixture onto seedlings at the three-leaf stage. Seedlings of susceptible (Thatcher) and resistant (Tc-*LrW*, or Tc-*LrW2*) checks were co-inoculated with hybrid seedlings. After allowing the oil to evaporate for 30 minutes inoculated seedlings were placed overnight in a dew chamber (Percival, model I60D, Perry, Iowa), with the following chamber conditions; water reservoir 30°C, chamber wall 5°C, and air

temperature 20°C. At 12 days post-inoculation seedlings were classified as resistant or susceptible based on their infection type. For all rust infection type scores the scale outlined in McIntosh *et al.*(1995), which is based on Stakman *et al.* (1962), was used (Table 1). If infection types were ambiguous (intermediate) inoculated leaves were removed and the plants were inoculated a second time after new leaves emerged.

Determining chromosome deficiencies in hybrids derived from haploids

For each population, Tc-*LrW* (haploid)/AC Foremost, and Tc-*LrW2* (haploid)/AC Foremost, 58 microsatellite markers were screened for polymorphism between their respective parents. Polymorphic microsatellite markers were generally common to both populations because of similarities in their parentage. DNA was extracted from lyophilized young leaf tissue of leaf rust susceptible hybrid plants. Tissue was macerated by placing 3 glass beads with the tissue into 1.5 ml microcentrifuge tubes and shaking with a modified paint shaker. Extraction buffer (0.11M Tris/HCl, 0.055M EDTA, 1.54M NaCl, 1.1% CTAB, and 2mg/sample proteinase K; buffer at 65°C) was mixed vigorously with the tissue (400µl buffer per sample). Then 44µl of 20% SDS was added to each tube, and the mixture was incubated at 65°C for 1.5 hours, mixing by inversion every 30 minutes. Next 400µl of chloroform:isoamyl alcohol (24:1) was added and the samples mixed by inversion for 20 minutes. After centrifuging the samples for five minutes, at 15800 x g, the supernatant was transferred to a fresh tube. DNA was precipitated by adding 200µl of isopropanol to each tube and mixed gently by inversion for about 5 minutes. Samples were centrifuged for five minutes at 15800 x g, followed by the removal of the supernatant. Pellets of DNA were washed by adding 1ml of cold

Table 1 – Definition of infection type ratings for leaf rust pustules on wheat leaves.

Infection type	Description
0	No uredia present visible
;	Necrotic flecks with no sporulation
1	Small uredia with necrosis
2	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis
3	Medium sized uredia (perhaps with modest amounts of chlorosis)
4	Large uredia with no chlorosis or necrosis

70% ethanol to each tube. Again, samples were centrifuged for five minutes at 15800 x g and the supernatant removed. After the pellets were air-dried, DNA was resuspended in 100µl of sterile water, and 2µl of RNAse (10µg/ml) was added to each tube. DNA samples were stored at -20°C.

For the first leaf rust susceptible hybrid found for each population at least one microsatellite marker per chromosome was used to determine the transmission of each of the 21 wheat chromosomes from both AC Foremost, and Tc-*LrW* or Tc-*LrW2*, to identify chromosome deficiencies (chromosomes, in whole or in part, not transmitted). Chromosomes that appeared to be deficient in the first leaf rust susceptible hybrids, based on failed transmission of microsatellite alleles from the haploid parent (Tc-*LrW* or Tc-*LrW2*), were tested with additional microsatellite markers, in intervals allowing more complete chromosome coverage, with all leaf rust susceptible hybrids from that cross to find which chromosome regions were deficient, and to see if the deficiencies were common to all susceptible hybrids. An Applied Biosystems (Foster City, CA, USA) thermocycler was used to perform all PCR reactions (PCR buffer 1x, dNTPs 0.2mM each, $MgCl_2$ 1.5 mM, primers 10 pmol each, Taq DNA polymerase 1 U, and approximately 50 ng genomic DNA; 2 min. 94°C, then 1 min. 95°C, 1 min 61°C, and 50 sec. 73°C for 30 cycles, followed by 5 min. 73°C). PCR products were run on 5% denaturing polyacrylamide gels in TBE buffer (0.089M tris, 0.089M boric acid, 0.050M EDTA) at 85 W for 2 hours, and visualized with silver staining (Promega, Madison, WI, USA), following the manufacturers protocol.

Screening for markers linked to LrW, *and* LrW2

Two F_2 populations were produced, one segregating for *LrW*, the other segregating for *LrW2*, by allowing resistant hybrids (from above populations), two plants per population, to self-pollinate. Three hundred and ninety Tc-*LrW*/AC Foremost and 391 Tc-*LrW2*/AC Foremost F_2 seedlings were inoculated with *P. triticina* as above and grown in a growth cabinet (16h light at 18°C, 8h dark at 17°C). Leaf rust susceptible individuals were identified and their DNA extracted. Microsatellite markers located on chromosomes that haploid deficiency mapping identified as the carriers of *LrW* and *LrW2* were screened for polymorphism between the parents in each population (PCR conditions as above). Markers that were polymorphic between the parents were tested on with leaf rust susceptible F_2 plants to test for linkage between microsatellite markers and *LrW,* and *LrW2.* The segregation of each marker was analyzed with a chi-squared test to see if the ratio of AC Foremost microsatellite alleles to Tc-*LrW*, or Tc-*LrW2*, microsatellite alleles differed significantly from the 1:1 ratio expected for single, unlinked genes. If linkage between a microsatellite marker and *LrW*, or *LrW2*, was observed, percent recombination was calculated by dividing the number of Tc-*LrW*, or Tc-*LrW2,* alleles by the total number of alleles, and multiplying by 100.

Assessing gene effectiveness

Seedlings of Tc-*LrW* and Tc-*LrW2* were inoculated at the two to three-leaf stage with 29 different *P. triticina* isolates using the technique described above. Seedlings were grown in soil filled flats in the greenhouse at 20 ± 4°C, with supplemental lighting provided by high-pressure sodium bulbs. Of the 29 isolates, there were 23 different

virulence phenotypes, as three different isolates of both MBDS and TJBJ and two isolates of SBDG, were tested in order to confirm that *LrW* and *LrW2* showed uniform reactions to different isolates of the same virulence phenotype. In addition an epidemic mix was tested, which included a representative, and proportional sample of the majority of the *P. triticina* virulence phenotypes found in western Canada in 2000 (McCallum and Seto-Goh, 2003). After 12 days post-inoculation seedlings were scored (Table 1).

Temperature sensitivity test

Seedlings of Tc-*LrW* and Tc-*LrW2* were inoculated at the two to three-leaf stage with three different virulence phenotypes, TJBJ, BBB, and MBDS as previously described, using Thatcher as the susceptible check. After plants were inoculated they were grown in growth cabinets, 16h light, and 8h dark, with constant temperature. The five temperatures tested were 15°C, 18°C, 21°C, 24°C, and 30°C. Three different races were used to minimize the potential differences that temperature may impose on different *P. triticina* isolates. Seedlings grown at 15°C were rated 15 days post-inoculation, seedlings grown at 18°C, 21°C, and 24°C were rated 12 days post-inoculation, and seedlings grown at 30°C were rated 10 days post-inoculation. The number of days between inoculation and rating differed because extreme temperatures slowed or accelerated uredial development.

4. Results

4.1 Mapping *LrW*

Generating and pollinating haploids of Tc-LrW

About 50 to 60 haploid wheat plants were produced. The first 20 vigorous plants were selected for crossing. From the 20 haploid plants of Tc-*LrW*, 455 heads, and 6968 spikelets, were pollinated with AC Foremost. In total 540 seeds were produced from these crosses. This translates into 1.19 seeds per head pollinated, and 0.08 seeds per spikelet pollinated.

Testing for leaf rust susceptible hybrids

Of 440 Tc-*LrW* (haploid)/AC Foremost hybrids seeded 417 plants were produced (94.8% germination). Of these 417 plants tested with *P. triticina* virulence phenotype MBDS, five plants (1-109, 1-178, 1-280, 1-369, and 1-438) were susceptible to leaf rust (Figure 4), the rest of the plants (412) were resistant. The resistant hybrids, though heterozygous for *LrW*, had similar infection types as the homozygous Tc-*LrW* checks, which was between ';1' and '1+', demonstrating the dominance of *LrW*. The frequency of leaf susceptible plants recovered in this population was 1.2% (5/417 x 100).

Determining chromosome deficiencies in hybrids derived from haploids

The first susceptible hybrid recovered, plant 1-109, was analyzed with 21 microsatellite markers, one marker per chromosome (Table 2), which were a subset of markers that were found to be polymorphic between AC Foremost and Tc-*LrW* (Table 3). The Tc-*LrW* allele of gwm397 on chromosome 4A was not present in plant 1-109 (Table

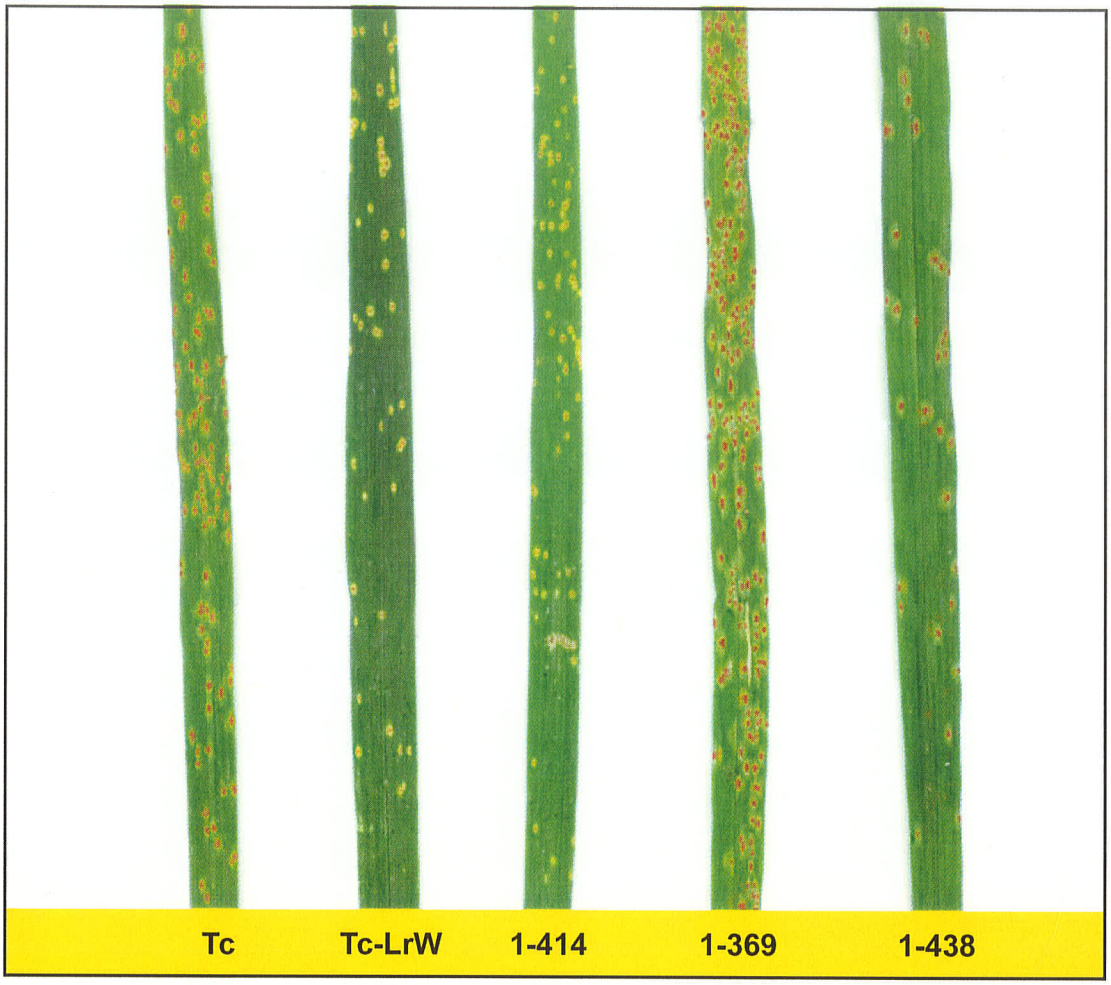

Figure 4 – Leaf rust pustules found on the second leaf of hybrid seedlings from the Tc-*LrW* (haploid)/AC Foremost population 12 days after inoculation with *P. triticina* virulence phenotype MBDS. Plants 1-369 and 1-438 are susceptible, while 1-414 is an example of a resistant hybrid. Tc (Thatcher) and Tc-*LrW* are the susceptible and resistant checks respectively.

Table 2 - Microsatellite allele transmission in plant 1-109, the first leaf rust susceptible hybrid found in the Tc-*LrW* (n)/ AC Foremost cross, and 2-39, the first leaf rust susceptible hybrid found in the Tc-*LrW2* (n)/ AC Foremost cross.

		Plant 1-109		Plant 2-39	
Marker[a]	Chr.[b]	Transmission of AC Foremost allele	Transmission of Tc-*LrW* allele	Transmission of AC Foremost allele	Transmission of Tc-*LrW2* allele
gwm136	1A	Y[c]	Y	Y	Y
gwm413	1B	Y	Y	Y	Y
gwm642	1D	Y	Y	Y	N[d]
gwm372	2A	Y	Y	Y	N
gwm148	2B	Y	Y	Y	Y
gwm030	2D	Y	Y	Y	Y
gwm674	3A	Y	Y	Y	Y
gwm493	3B	Y	Y	Y	Y
gwm383	3D	Y	Y	-	-
gwm645	3D	-[e]	-	Y	N
gwm397	4A	Y	N	Y	Y
gwm368	4B	Y	Y	Y	Y
gdm125	4D	Y	Y	Y	Y
gwm156	5A	Y	Y	Y	Y
gwm159	5B	Y	Y[f]	Y	N
gwm190	5D	Y	Y	Y	Y
gwm570	6A	Y	Y	Y	Y
gwm219	6B	Y	Y	Y	Y
gwm325	6D	Y	Y	Y	Y
gwm332	7A	Y	Y	Y	Y
gwm537	7B	Y	Y	Y	Y
gwm295	7D	Y	Y	Y	N

[a] All 'gwm' microsatellite markers are from Röder *et al.* 1998, all 'gdm' microsatellite markers are from Pestova, 2000.
[b] Chr. is an abbreviation for chromosome.
[c] 'Y' indicates that the microsatellite marker was polymorphic.
[d] 'N' indicates that the microsatellite marker was monomorphic.
[e] Indicates that marker was not used.
[f] This marker was initially scored as a false positive in plant 1-109.

Table 3 – List of microstellite markers screened for polymorphism between AC Foremost and Tc-*LrW*, and their results.

Marker	Chromosome	Polymorphic between AC Foremost and Tc-*LrW*	Reference
gwm135	1A	N[a]	Röder *et al.* 1998
gwm136	1A	Y[b]	Röder *et al.* 1998
gwm153	1B	Y	Röder *et al.* 1998
gwm413	1B	Y	Röder *et al.* 1998
gwm106	1D	N	Röder *et al.* 1998
gwm642	1D	Y	Röder *et al.* 1998
gwm312	2A	N	Röder *et al.* 1998
gwm372	2A	Y	Röder *et al.* 1998
gwm120	2B	N	Röder *et al.* 1998
gwm148	2B	Y	Röder *et al.* 1998
gwm257	2B	N	Röder *et al.* 1998
gwm501	2B	N	Röder *et al.* 1998
gwm630	2B	N	Röder *et al.* 1998
gwm030	2D	Y	Röder *et al.* 1998
gwm102	2D	N	Röder *et al.* 1998
gwm157	2D	N	Röder *et al.* 1998
gwm539	2D	N	Röder *et al.* 1998
gwm155	3A	N	Röder *et al.* 1998
gwm162	3A	N	Röder *et al.* 1998
gwm369	3A	N	Röder *et al.* 1998
gwm391	3A	N	Röder *et al.* 1998
gwm480	3A	N	Röder *et al.* 1998
gwm674	3A	Y	Röder *et al.* 1998
gwm108	3B	Y	Röder *et al.* 1998
gwm493	3B	Y	Röder *et al.* 1998
gwm161	3D	N	Röder *et al.* 1998
gwm383	3D	Y	Röder *et al.* 1998
gwm004	4A	N	Röder *et al.* 1998
gwm160	4A	N	Röder *et al.* 1998
gwm397	4A	Y	Röder *et al.* 1998
gwm601	4A	N	Röder *et al.* 1998
gwm610	4A	N	Röder *et al.* 1998
gwm637	4A	N	Röder *et al.* 1998
gwm368	4B	Y	Röder *et al.* 1998
gwm538	4B	N	Röder *et al.* 1998
gdm125	4D	Y	Pestova *et al.* 2000

gdm129	4D	N	Pestova *et al.* 2000
gwm126	5A	Y	Röder *et al.* 1998
gwm156	5A	Y	Röder *et al.* 1998
gwm159	5B	Y	Röder *et al.* 1998
gwm554	5B	N	Röder *et al.* 1998
gwm182	5D	N	Röder *et al.* 1998
gwm190	5D	Y	Röder *et al.* 1998
gwm494	6A	N	Röder *et al.* 1998
gwm570	6A	Y	Röder *et al.* 1998
gwm219	6B	Y	Röder *et al.* 1998
gwm613	6B	N	Röder *et al.* 1998
gwm323	6D	Y	Röder *et al.* 1998
gwm469	6D	N	Röder *et al.* 1998
gwm060	7A	N	Röder *et al.* 1998
gwm063	7A	N	Röder *et al.* 1998
gwm130	7A	N	Röder *et al.* 1998
gwm332	7A	P	Röder *et al.* 1998
gwm471	7A	N	Röder *et al.* 1998
gwm146	7B	N	Röder *et al.* 1998
gwm537	7B	Y	Röder *et al.* 1998
gwm037	7D	N	Röder *et al.* 1998
gwm295	7D	Y	Röder *et al.* 1998

[a] 'N' indicates that the microsatellite marker was monomorphic.
[b] 'Y' indicates that the microsatellite marker was polymorphic.

2), therefore Tc-*LrW* did not transmit chromosome 4A. This was confirmed in plant 1-109 by another microsatellite mapped to chromosome 4A, wmc161, as the Tc-*LrW* allele for this marker was also not present in plant 1-109 (Table 4). The next two leaf rust susceptible hybrids, plants 1-178 and 1-280, were tested with the same microsatellites as used in analysis of plant 1-109 (same markers listed in Table 2 for plant 1-109). These two plants did not show deficiencies for microsatellite markers chromosome 4A, or microsatellite markers on any other chromosome. All microsatellite markers tested appeared to be dimorphic in 1-178 and 1-280, meaning alleles of both AC Foremost and Tc-*LrW* was present in these plants. A closer look revealed that the microsatellite marker gwm159 used to detect chromosome 5B transmission was not clearly scored in this cross. Microsatellite markers on chromosomes 4A and 5B (Table 4) were tested to find deficiencies common to all three leaf rust susceptible hybrids (1-109, 1-178, and 1-280). It was found that that in addition for being dimorphic for gwm397 on chromosome 4A, plants 1-178 and 1-280 were dimorphic for wmc161 and wmc262, both on chromosome 4A (Table 4), indicating that no chromosome 4A deficiencies were detected in these plants, although 1-109 showed deficiencies with all of the above markers on chromosome 4A. However, plants 1-109, 1-178, and 1-280 all had deficiencies for microsatellite markers chromosome 5B. Evidently, plants 1-178 and 1-280 are monosomic for one chromosome (5B), but plant 1-109 is a double monosomic (4A and 5B). Further analysis showed that Tc-*LrW* failed to transmit alleles of gwm443, gwm133, gwm67, wmc149, wmc75 (Figure 5), and wmc235 (all on chromosome 5B) to all three susceptible hybrids (Table 4). However, the Tc-*LrW* allele of gwm497, also on chromosome 5B, was transmitted to plants 1-109, 1-178, and 1-280. The final two leaf rust susceptible hybrids

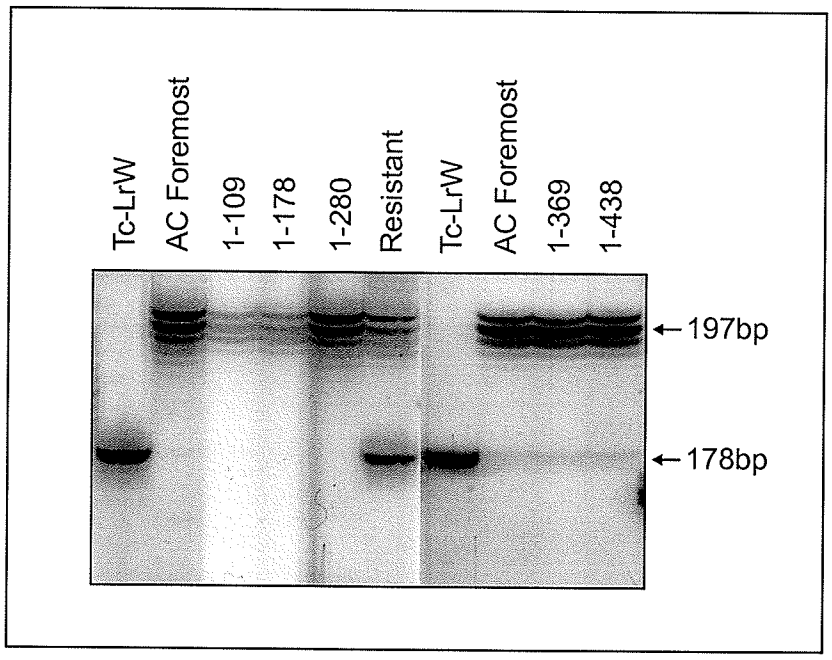

Figure 5 - Example of deficient microsatellite (wmc75) alleles in susceptible hybrids missing chromosome 5B from Tc-*LrW*. Plants 1-109, 1-178, 1-280, 1-369, and 1-438 are susceptible hybrids and have failed to receive the Tc-*LrW* allele of wmc75 (178 bp). An example of a resistant hybrid, in the lane labelled 'Resistant', did receive this allele. All hybrids received the AC Foremost wmc75 allele (197 bp) as expected.

Table 4 - Microsatellite allele transmission on chromosomes 4A and 5B in leaf rust susceptible hybrids from the Tc-*LrW* (n)/AC Foremost cross.

Marker[a]	Chromosome	Approximate distance to the centromere	Polymorphic between AC Foremost and Tc-*LrW*	Transmission of Tc-*LrW* allele in susceptible hybrids				
				1-109	1-178	1-280	1-369	1-438
wmc096	4AS	5cM	N[b]	-[c]	-	-	-	-
gwm601	4A	0cM	N	-	-	-	-	-
wmc161	4AL	40cM	Y[d]	N	Y	Y	-	-
wmc262	4AL	49cM	Y	N	Y	Y	-	-
gwm160	4AL	65cM	N	-	-	-	-	-
wmc219	4AL	67cM	N	-	-	-	-	-
wmc313	4AL	71cM	Y	N	Y	Y	-	-
gwm443	5BS	66cM	Y	N	N	N	N	N
gwm234	5BS	30cM	N	-	-	-	-	-
wmc149	5BS	26cM	Y	N	N	N	N	N
gwm133	5BS	09cM	Y	N	N	N	N	N
gwm554	5BS	07cM	N	-	-	-	-	-
gwm067	5BL	06cM	Y	N	N	N	N	N
gwm497	5BL	104cM	Y	Y	Y	Y	Y	Y
wmc075	5BL	55cM	Y	N	N	N	N	N
gwm408	5BL	64cM	N	-	-	-	-	-
wmc235	5BL	80cM	Y	N	N	N	N	N

[a] All 'gwm' microsatellite markers are from Röder *et al.* 1998, all 'wmc' microsatellite markers are from Somers, 2003.
[b] 'N' means 'no'.
[c] indicates that this microsatellite marker was not used with that plant.
[d] 'Y' means yes.

identified were plants 1-369 and 1-438. These two plants showed the same chromosome deficiencies on chromosome 5B as described above for plants 1-109, 1-178, and 1-280. In summary, the transmission of chromosome 5B from Tc-*LrW* to the susceptible hybrids was negative for 5BS (short arm) and 5BL (long arm) proximal of wmc235, and positive distal of gwm497 on 5BL (Figure 6).

Screening for markers linked to LrW

The F_2 population segregating for *LrW* contained 390 individuals (307 resistant, 87 leaf rust susceptible), and fit the expected ratio for a single gene, 3 resistant (292.5):1 leaf rust susceptible (97.5) ($\chi^2 = 1.5$, $p = 0.22$). Nine microsatellite markers were tested for linkage to *LrW* on chromosome 4A (Table 5). No linkage was observed. However, when haploid deficiency mapping placed *LrW* on chromosome 5B with all leaf rust susceptible hybrids lacking the same segment of 5B, 10 leaf rust susceptible F_2 plants were screened with the microsatellite markers on chromosome 5B (Table 5). Microsatellite marker gwm443 and the *LrW* locus were linked, thus 46 individuals were tested (Figure 7 and Table 5). The total number of gwm443 alleles in the F_2 population was 92, as each of the 46 individuals contained two alleles. Of these 92 alleles, 13 were Tc-*LrW* alleles, and 79 were AC Foremost alleles, which deviated significantly ($\chi^2 = 47.3$, $p = 5.9 \times 10^{-12}$) from the value of 46 each expected if no linkage existed (1:1 ratio for a single gene). This resulted in an observed recombination of 14.1%. Among these progeny, two were homozygous for the Tc-*LrW* allele (gwm443), being double recombinants, nine were heterozygous, and 35 were homozygous for the AC Foremost

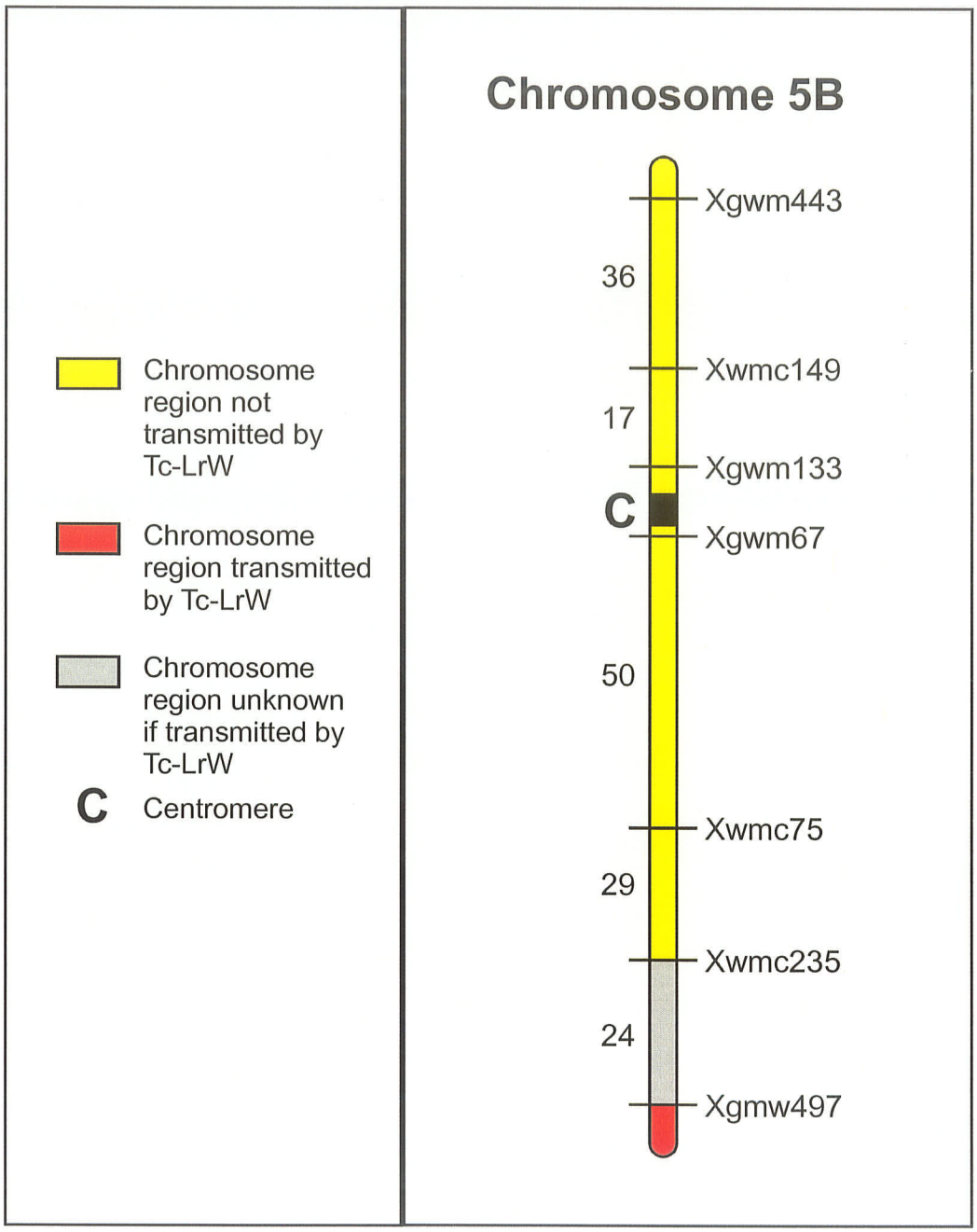

Figure 6 – Proposed model of Tc-*LrW* microsatellite allele transmission for chromosome 5B in all five susceptible hybrids (1-109. 1-178, 1-280, 1-369, and 1-438). The grey region exists because we cannot determine the precise break point, but this break point is within this region.

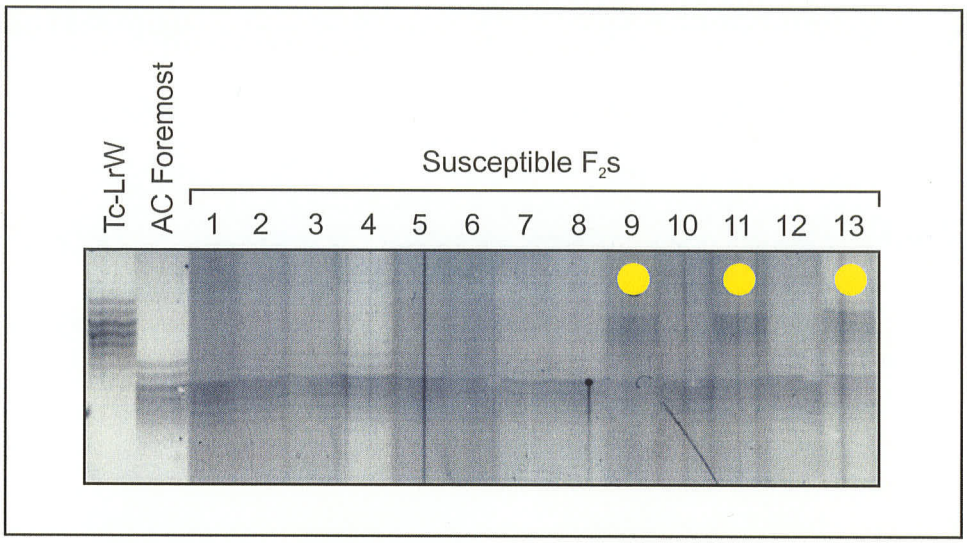

Figure 7 - Susceptible F_2 progeny from the cross Tc-*LrW* (haploid)/AC Foremost segregating for *LrW* tested with microsatellite marker gwm443 located on the distal portion of chromosome 5BS. Individuals 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 are homozygous for the AC Foremost allele of gwm443. Individuals 9, 11, and 13 (marked with the yellow dots) are heterozygous, thus each of these plants has one chromosome that has had a recombination event occur between *lrW* and gwm443.

Table 5 - Segregation of microsatellite alleles on chromosomes 4A and 5B in leaf rust susceptible F_2 plants derived from two self-pollinated hybrids from Tc-*LrW* (n)/AC Foremost.

Marker[a]	Chromosome	Distance to the centromere	Polymorphic between AC Foremost and Tc-*LrW*	Run with F_2	Number of AC Foremost alleles	Number of Tc-*LrW* alleles	Total number of alleles	Chi-squared test for 1:1 ratio[b]	p-value of Chi-squared test
gwm4	4AS	6cM	N[c]						
wmc96	4AS	2cM	Y[d]	Y	9	11	20	0.20	0.65
gwm601	4AS	1cM	Y						
wmc173	4A	0cM	Y	Y	10	10	20	0.00	1.00
wmc89	4AL	3cM	Y						
wmc15	4AL	4cM	N						
gwm44	4AL	5cM	N						
gwm165	4AL	5cM	Y	Y	10	10	20	0.00	1.00
wmc516	4AL	12cM	N						
gwm397	4AL	18cM	Y	Y	11	9	20	0.20	0.65
wmc513	4AL	20cM	N						
gwm565	4AL	27cM	N						
gwm637	4AL	33cM	Y	Y	8	12	20	0.80	0.37
wmc161	4AL	42cM	Y	Y	10	10	20	0.00	1.00
wmc262	4AL	51cM	Y	Y	8	10	18[e]	0.11	0.74
wmc283	4AL	54cM	Y						
wmc232	4AL	60cM	Y						
wmc500	4AL	62cM	Y						
gwm160	4AL	78cM	Y	Y	11	9	20	0.20	0.65
wmc219	4AL	80cM	Y						
wmc497	4AL	83cM	N						
wmc313	4AL	84cM	Y	Y	8	10	18	0.11	0.74
gwm443	5BS	66cM	Y	Y	18	2	20	12.80	0.03

gwm443[f]	5BS	66cM	Y	Y	79	13	92	47.35	5.9×10^{-12}
wmc149	5BS	26cM	Y	Y	11	9	20	0.20	0.65
gwm133	5BS	9cM	Y	Y	12	8	20	0.80	0.37
gwm67	5BL	6cM	Y	Y	10	8	18	0.11	0.74
wmc75	5BL	55cM	Y	Y	9	11	20	0.20	0.65
wmc235	5BL	80cM	Y	Y	8	12	20	0.80	0.37

[a] All 'gwm' microsatellite markers are from Röder *et al.* 1998, all 'wmc' microsatellite markers are from Somers, 2003.
[b] Single gene ratio is expected to be 1:1 if no linkage exists between the marker and *lrW*.
[c] 'N' means no.
[d] 'Y' means yes.
[e] Only 18 alleles present due to a failed reaction.
[f] This marker (gwm443) was run on a larger population (46 individuals) to confirm linkage observed on the smaller population (20 individuals).

allele. No linkage with *LrW* was found with any other microsatellite marker on chromosome 5B (Table 5).

4.2 Mapping *LrW2*

Generating and pollinating haploids

The first 20 vigorous haploid wheat plants from the 50 to 60 produced were selected for crossing. There was a total of 355 heads from Tc-*LrW2* haploids, with a combined total of 5429 spikelets, pollinated by AC Foremost. From these crosses, 531 seeds were produced. The crossing success rates were 1.50 seeds per head, and 0.10 seeds per spikelet.

Testing for leaf rust susceptible hybrids

There were 488 plants produced from the 531 seeds (91.2% germination) of the Tc-*LrW2* (haploid)/AC Foremost cross. Inoculating hybrids as young seedlings was not informative because all of the hybrids had infection types similar to Thatcher, the susceptible check, and were more susceptible than the homozygous resistant checks (Tc-*LrW*). After inoculating a second time at a later growth stage the symptoms on penultimate leaves clearly distinguished resistant from susceptible hybrids (Figure 8). There were four leaf rust susceptible hybrids (2-39, 2-271, 2-360, and 2-397), thus recovery of susceptible plants was 0.82%.

Figure 8 - Leaf rust pustules found on penultimate leaves of hybrid plants from the Tc-*LrW* (haploid)/AC Foremost population 12 days after inoculation with *P. triticina* virulence phenotype MBDS. Plant 2-360 is a susceptible hybrid with pustules very similar to Tc (Thatcher), the susceptible check. Plant 2-94 is an example of a resistant hybrid, and appears similar to the resistant check (not shown).

Determining chromosome deficiencies in hybrids derived from haploids

Plant 2-39 had its chromosome constitution tested with microsatellite markers first, and deficiencies were found for chromosomes 2A, 5B, 1D, and 7D, as determined by gwm372, gwm159, gwm642, and gwm295 respectively (Table 2). Deficiencies were shown by the absence of the Tc-*LrW2* microsatellite allele in the hybrid, but in all hybrids the AC Foremost allele was present (Figure 9).

The next three susceptible hybrids, 2-271, 2-360, and 2-397, which were identified later due to staggered planting, were screened with microsatellite markers that showed deficiencies in plant 2-39. These three plants showed deficiencies in chromosome 1D from Tc-*LrW2* as determined by marker gwm642 (Table 6). However, the later three plants had no detected deficiencies for chromosomes 2A, 3D, 5B, and 7D. Since plant 2-39 clearly was deficient for all the markers tested on chromosome 2A, and two of three markers on 1D (Table 6), plants 2-271, 2-360, and 2-397 were tested extensively for deficiencies on these two chromosomes. It was found that all four leaf rust susceptible hybrids had deficiencies on chromosome 1D, but only 2-39 had chromosome 2A deficiencies (Table 6). The size of chromosome 1D deficiencies from Tc-*LrW2* in the leaf rust susceptible hybrids varied between individuals. The Tc-*LrW2* allele of gdm126 was present in plants 2-39 and 2-360 (Figure 10 and Table 6). Furthermore, the Tc-*LrW2* allele of gwm642 was present only in plant 2-360. Although no microsatellite markers distal of gwm337 on 1DS were available to test transmission of Tc-*LrW2* alleles, it is unlikely that any segments of 1DS were transmitted to plants 2-39 and 2-360 from Tc-*LrW2*. No Tc-*LrW2* microsatellite alleles on chromosome 1D were detected in plants 2-271 and 2-397 (Figure 10 and Table 6).

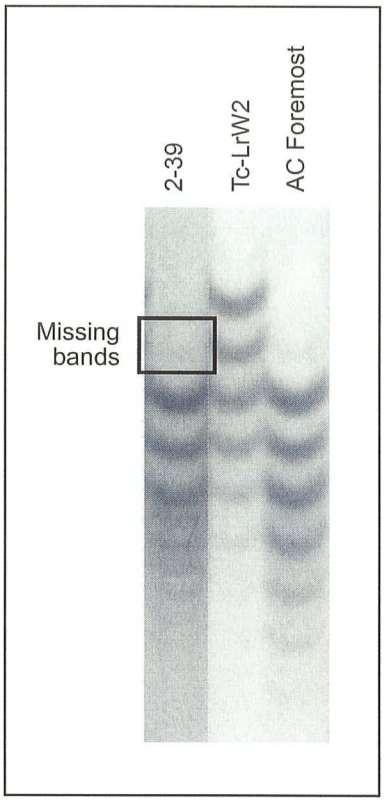

Figure 9 - Example of a deficient microsatellite (gwm642) allele in a susceptible hybrid from cross Tc-*LrW2* (haploid)/AC Foremost. Susceptible hybrid 2-39 did not receive the Tc-*LrW2* allele of gwm642 (chromosome 1D).

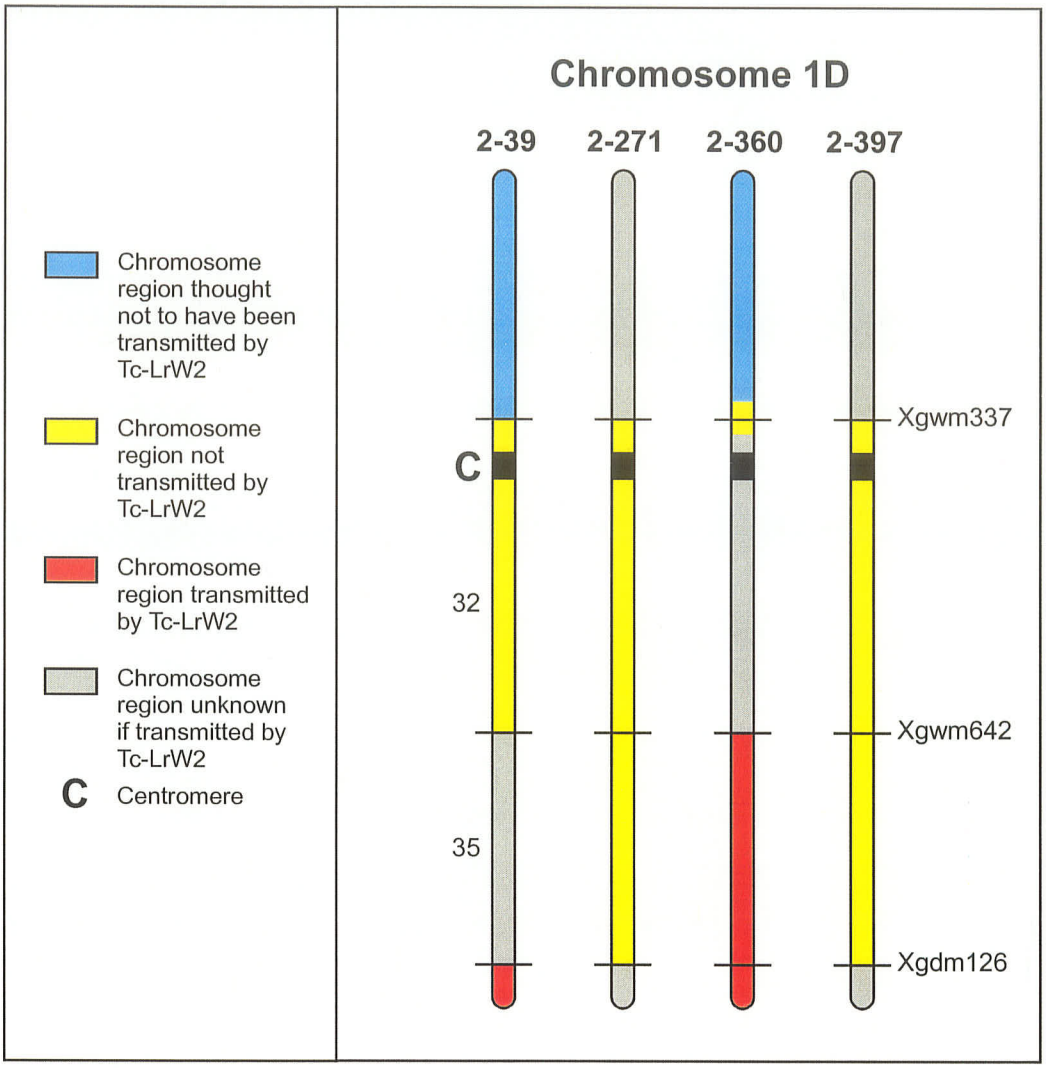

Figure 10 – Proposed model of Tc-*LrW2* microsatellite allele transmission for chromosome 1D in four susceptible hybrids from the cross Tc-*LrW2* (haploid)/AC Foremost. The blue region is believed not to have been transmitted by Tc-*LrW2* because the red region indicates that the long arms paired during meiosis. Since it is unlikely that both arms would pair with a homoeologous chromosome, it is most likely that these regions were not transmitted to susceptible hybrids.

Table 6 - Microsatellite allele transmission on chromosomes 1D, 2A, 5B, and 7D in leaf rust susceptible hybrids from the Tc-*LrW2* (n)/AC Foremost cross

				Transmission of Tc-*LrW2* allele in susceptible plants			
Marker[a]	Chromosome	Approximate distance to the centromere	Polymorphic between AC Foremost and Tc-*LrW*	2-39	2-271	2-360	2-397
gwm147	1DS	34cM	N[b]	-[c]	-	-	-
gwm106	1DS	10cM	N	-	-	-	-
gwm337	1DS	3cM	Y[d]	N	N	N	N
gwm642	1DL	29cM	Y	N	N	Y	N
gdm126	1DL	73cM	Y	Y	N	Y	N
gwm232	1DL	79cM	N	-	-	-	-
wmc407	2AS	47cM	Y	N	Y	Y	Y
wmc177	2AS	29cM	Y	N	Y	Y	Y
wmc522	2AS	10cM	Y	N	Y	Y	Y
gwm265	2AL	46cM	Y	Y	Y	Y	Y
gwm311	2AL	48cM	N	-	-	-	-
gwm443	5BS	66cM	Y	Y	-	-	-
gwm159	5BS	06cM	Y	N	Y	Y	Y
wmc537	5BL	25cM	Y	Y	-	-	-
wmc160	5BL	74cM	Y	Y	-	-	-
gwm295	7DS	21cM	Y	N	Y	Y	Y

[a] All 'gwm' microsatellite markers are from Röder *et al.* 1998, all 'wmc' microsatellite markers are from Somers, 2003, and all 'gdm' microsatellite markers are from Pestova *et al.* 2000.

[b] 'N' means no.

[c] Indicates that marker was not used.

[d] 'Y' means yes.

Table 7 – Segregation of microsatellite alleles on chromosome 1D in leaf rust susceptible F_2 plants derived from two self-pollinated hybrids from Tc-*LrW2* (n)/AC Foremost.

Marker[a]	Chr.[b]	Distance to the centromere	Polymorphic between AC Foremost and Tc-*LrW*	Run with F_2	Number of AC Foremost alleles	Number of Tc-*LrW* alleles	Total number of alleles	Chi-squared test for 1:1 ratio[c]	p-value of Chi-squared test
wmc336	1DS	25cM	Y[d]	Y	11	9	20	0.20	0.65
gwm337	1DS	03cM	Y	Y	13	7	20	0.80	0.18
gwm337[e]	1DS	03cM	Y	Y	29	27	56	0.07	0.79
wmc429	1DL	07cM	Y	Y	12	8	20	0.80	0.37
gwm642	1DL	29cM	Y	Y	8	8	16	0.00	1.00

[a] all 'gwm' microsatellite markers are from Röder *et al.* 1998, all 'wmc' microsatellite markers are from Somers, 2003, and all 'gdm' microsatellite markers are from Pestova *et al.* 2000.

[b] Chr. is an abbreviation for chromosome.

[c] Single gene ratio is expected to be 1:1 if no linkage exists between the marker and *lrW2*.

[d] 'Y' means yes.

[e] This marker was run twice because the smaller sample size of 20 showed a low p-value, therefore a larger population was used to test for distant linkage.

Screening for markers linked to LrW2

An F_2 population of 391 plants segregating for *LrW2* (301 resistant and 90 leaf rust susceptible), when inoculated with *P. triticina* virulence phenotype MBDS, fit the expected 3 resistant (293.3) : 1 leaf rust susceptible (97.7) ratio ($\chi^2 = 0.82$, $p = 0.37$) for a single gene. A subset of 10 leaf rust susceptible F_2 plants, thus assaying 20 alleles, was tested with four microsatellite markers (Table 7) spaced evenly along chromosome 1D. One marker, gwm337, showed possible linkage, as only seven of the 20 alleles were from Tc-*LrW2* (35%) instead of the expected 10 alleles (50%). To get a better linkage estimate between the *LrW2* locus and gwm337, 30 susceptible F_2 plants, thus 60 alleles, were tested with gwm337. In addition wmc336 was also run with these individuals, since this marker is distal to gwm337 (on the short arm of 1D), and no markers on the long arm of chromosome 1D showed linkage to *LrW2*. No linkage was found between any microsatellite markers tested on 1D and *LrW2*. The utility of markers distal of gwm337 on 1DS was poor because of complex electrophoretic patterns.

4.3 Effectiveness of *LrW* and *LrW2*

Tc-*LrW* was resistant to all rust isolates tested, with infection types ranging from ';' to '1+' (Table 8). The most common infection type was ';1'. All of the infection types for Tc-*LrW* would be considered to be low, or resistant, infection types. Different isolates of the same virulence phenotype did not have a differential reaction on *LrW*.

Table 8 - Infection types on Tc-*LrW* and Tc-*LrW2* using 29 different isolates of *P. triticina*.

Isolate[a]	Virulence Phenotype[b]	Tc-*LrW*	Tc-*LrW2*	Tc
	MBRJ	;1[c]	;11+	4
	SBDG	;1	;1	4
	CBDJ	;1--	;1	34
	MGBJ	;11+	12	3+4
	PBDG	;1	12-	3+4
	BBB	;1	;1	3+4
	PCLR	;1--	12	34
12-3	*MBDS*	;1	;12	34
	TJBJ	;1	;12	3+4
	NBBR	;11-	11+	34
00-52-2	*MCPS*	;1-	;1-	4
00-44-2	*TCMJ*	;1	12	4
00-148-2	*SBDG*	;11+	;11-	3+
99-93-1	*TFRJ*	;11+	12	34
00-32-1	*TJBJ*	;1	;11-	3+4
00-13-1	*MBDS*	;1-1--	;1-	3+
00-74-1	*SGBJ*	;1+-	1+	3+
00-24-1	*TBPS*	;11-	;1	3+4
99-46-2	*MDRJ*	;11-	;11+	3+
99-228-1	*PBMR*	;1	1+2	3+4
00-30-2	*TJBJ*	;1	12	3+4
99-8-1	*TFMJ*	;1	12	3+4
00-30-1	*THMJ*	;11+	12	4
2001	*Epidemic*[d]	;11-	;1	3+4
00-7-2	*MBDS*	;1-	;1	4
99-127-1	*MFMJ*	;11-	;1	3+4
00-179-1	*THBJ*	;1-	;1	4
00-53b-1	*TGLJ*	;11-	;1	3+
99-231-2	*PBLR*	;11-	11+	3+4

[a] Not all virulence phenotypes have an isolate number.
[b] Nomenclature as described by Long and Kolmer, 1989, and McCallum and Seto-Goh, 2003.
[c] Infection types as described in Table 1.
[d] Epidemic mixture is representative of virulence phenotypes found in western Canada in 2000 (McCallum and Seto-Goh, 2003).

No virulence was found on Tc-*LrW2* with any of the isolates used (Table 8). Infection types ranged from ';' to '2', and all infection types are considered resistant (McIntosh *et al.* 1995). Only a few isolates resulted in a '12' infection type and were generally not variable. There were no differential reactions observed when Tc-*LrW2* was inoculated with different isolates of the same virulence phenotype.

4.4 Temperature sensitivity of *LrW* and *LrW2*

Infection types were the lowest (';' to '1') on Tc-*LrW* at 18°C compared to any other temperature (Table 9). The infection types at 15°C were moderate/low ('1+' to '2+'), and after 18°C there was increasing infection types as temperature increased (';' to '2+'). At 30°C the infection types were approximately the same as at 15°C. It should be noted that at higher temperatures infection types were determined two days earlier than the normal 12 days because of accelerated uredia development on the check line Thatcher. Different virulence phenotypes had little differentiation for infection type, except at 21°C, where BBB had somewhat higher infection types compared to MBDS and TJBJ.

The infection types on Tc-*LrW2* were quite low (';' to '1+') at both 15°C and 18°C (Table 9). Starting at 21°C there was an increase in infection type severity ('1' to '2+') as temperature increased. At 21°C the infection type was low/moderate, however an intermediate reaction ('2' to '2+', except BBB was '1' to '2') was seen at both 24°C and 30°C. The effect of different virulence phenotypes of *P. triticina* on infection type appeared to not be significant, except that BBB at 24°C and 30°C had reduced infection type compared MBDS and TJBJ (Table 9).

Table 9 - Infection types on Tc-*LrW* and Tc-*LrW2* at five different incubation temperatures with three different virulence phenotypes of *P. triticina*.

Temperature	Virulence Phenotype[a]	Tc-*LrW*	Tc-*LrW2*	Thatcher
15°C	TJBJ	1+-[b]	;1-	4
	MBDS	1+22-	;1+-	4
	BBB	1+2+-	1-	4
18°C	TJBJ	;1	;1	3+
	MBDS	;1-	;1	3+
	BBB	;1-	;1	3+4
21°C	TJBJ	12	12	3+
	MBDS	12	12	4
	BBB	22+	122+	4
24°C	TJBJ	12c	2+	3+
	MBDS	2c	22+	3+
	BBB	122+	12	3+
30°C	TJBJ	;122+	22+	3
	MBDS	;1	22+	3
	BBB	1+-2	12	3+

[a] Nomenclature as described by Long and Kolmer, 1989, and McCallum and Seto-Goh, 2003.

[b] Infection types as described in Table 1.

5. Discussion

Pollinating haploids

The success rates from pollinating haploids, in terms of relative seed set, did not greatly differ between haploids of Tc-*LrW* and Tc-*LrW2*. Sears (1939) recovered 14 seeds from a haploid wheat plant pollinated with euploid wheat. It was reported that less than 300 florets were pollinated. If we estimate, on average, three synchronously fertile florets per spikelet, then this equals 100 spikelets if the actual number of florets was close to 300. That works out to an approximate seed set frequency of at least 0.14 seeds per spikelet. This is greater than the 0.10 seeds per spikelet recovered from Tc-*LrW2* haploids, and 0.08 seeds per spikelet recovered from Tc-*LrW* haploids. All of the above values are greater than the success rate reported by Thomas *et al.* (2001), where one seed was produced for every four heads pollinated, which can be estimated to be 0.02 seeds per spikelet pollinated. There is apparent variability between wheat genotypes in their ability to set seed as haploids when pollinated by euploid wheat, which can be attributed to genetic factors. In an ongoing study (Hiebert, Thomas, and McCallum, unpublished data) haploids of the cultivar Marquis produced seed at a much lower frequency than the above studies. However, when haploids of Marquis x Little Club hybrids were pollinated seed set had an estimated five-fold increase as compared to Marquis. Furthermore, Sears (1939) found a haploid wheat plant that was completely female sterile in addition to its normal male sterility. This is a noteworthy consideration when choosing genetic backgrounds for implementing haploid deficiency mapping, however this would seem difficult to predict. For example, Marquis and Thatcher, Thatcher accounted for most of the genetic background of the haploids (Tc-*LrW* and Tc-*LrW2*) in this study, have a high

degree of relatedness (DePauw and Hunt, 2001). Despite this it seems that their respective ability to set seed as haploids is very different.

Testing for leaf rust susceptible hybrids

Leaf rust susceptible hybrids derived from Tc-*LrW* haploids were easily identified at the seedling stage. The resistant hybrids, despite heterozygosity for *LrW*, had infection types very similar to the homozygous resistant check, Tc-*LrW*. In contrast, identifying susceptible hybrids derived from Tc-*LrW2* haploids at the seedling stage proved difficult. This difficulty was due to the relatively susceptible infection types of the resistant hybrids at the seedling stage, which were similar to Thatcher, the susceptible check. Some variation was seen in resistant hybrid seedling reactions to rust, ranging from intermediate (2, 2+) to high (3+). However, when these plants were inoculated at a more mature stage the resistant hybrids had a much lower infection type, and closely resembled the homozygous resistant check Tc-*LrW2*. It appeared as though *LrW2* is incompletely dominant at the seedling stage, but complete dominance is found at more mature plant stages. It is common for genes to be more effective at the adult stage (e.g. adult resistance genes) (Dyck and Kerber, 1985). Dyck (1994) did not report any observed incomplete dominance, but rather *LrW2* was simply referred to as a seedling resistance gene.

Determining chromosome deficiencies in hybrids derived from haploids

The data showed that *LrW* was on chromosome 5B, as all susceptible plants did not receive most of the Tc-*LrW* microsatellite alleles found on 5B (Table 4 and Figure 6).

The transmission of chromosome 5B from Tc-*LrW* was identical in all five leaf rust susceptible hybrids, at least to level of resolution possible with current molecular markers. In each of the five susceptible hybrid gwm497 (chromosome 5B) was transmitted from Tc-*LrW*, but more importantly the deficiencies, gwm443, wmc149, gwm133, gwm67, gwm75, and gwm235 (all on chromosome 5B), were common to all susceptible plants. Furthermore, no other chromosome deficiencies were common to the susceptible hybrids. Plant 1-109 was a double monosomic (4A and 5B), but plants 1-178 and 1-280 were both disomic for each chromosome except for 5B. The last two susceptible hybrids found, 1-369 and 1-438, were only screened for chromosome 5B deficiencies only to confirm the data collected from the first three plants. Both plants 1-369 and 1-438 had the same deficiencies as plants 1-109, 1-178, and 1-280. Since the only selection criterion for these plants was their leaf rust susceptibility, it is highly unlikely that these common chromosome deficiencies were due to chance, rather than being diagnostic of chromosome 5B carrying *LrW*.

The chromosome deficiencies were not as consistent between leaf rust susceptible hybrids in the Tc-*LrW2* (haploid)/AC Foremost population compared to the Tc-*LrW* (haploid)/AC Foremost population. The first leaf rust susceptible hybrid found, plant 2-39, had several apparent deletions (Table 2) in addition to monosomy for chromosomes 2A and 1D (Table 6). However, all four susceptible hybrids in the Tc-*LrW2* (haploid)/AC Foremost population (2-39, 2-271, 2-360, and 2-397) did not receive the Tc-*LrW2* gwm337 allele on chromosome 1D (Figure 10). There was variation in the number of Tc-*LrW2* microsatellite alleles on chromosome 1D inherited by the four

susceptible hybrids. However, there is agreement that at least part of chromosome 1D is deficient in all four hybrids indicating that *LrW2* is on chromosome 1D.

The short arm of chromosome 1D distal of gwm337 was thought not to have been transmitted by Tc-*LrW2* in plants 2-39 and 2-360 because transmitted segments of otherwise deficient chromosomes are thought to be caused by homoeologous exchange during haploid meiosis (Figure 10). Since both 2-39 and 2-360 have had a putative homoeologous recombination event on the long arm of chromosome 1D, it is unlikely that the short arm also paired and recombined, despite the lack of marker evidence. The rationale for this is that the mean frequency of chiasma per bivalent in haploid wheat has been shown to equal is one chiasma per bivalent (Jauhar *et al.* 1991). Therefore if the long arm paired during meiosis, evident by the transmission of Tc-*Lrw2* alleles on 1DL, it is improbable that the short arm would also pair and be transmitted, thus the region is assumed to be deficient in these leaf rust susceptible hybrids (Figure 10).

Screening for markers linked to LrW and LrW2

The microsatellite marker gwm443 showed linkage to the *lrW* (the alternate allele of *LrW* that conditions susceptibility) locus when tested with the susceptible progeny from the F_2 population segregating for *LrW*. According to Röder *et al.* (1998), gwm443 is the terminal microsatellite on the short arm of chromosome 5B (5BS). Thus, the conclusion is that *LrW* is on chromosome 5BS, 14.1 cM from gwm443 in this cross as determined by 92 meioses. The only other *Lr* gene known to be on chromosome 5B is *Lr18*, but it was been shown to be on the long arm (McIntosh *et al.* 1995). Therefore, *LrW* is a unique gene and should receive an official gene name.

Leaf rust susceptible F_2 plants were selected for mapping because the genotype at the resistance gene locus was known, *lrW/lrW,* allowing accurate determination of recombination between the gene and neighboring microsatellites. For example individual 1 in Figure 7 only has the AC Foremost gwm443 allele, which represents homozygosity at that locus. Thus no recombination was observed in the two meioses that occurred in the generation of that plant. Individual number 9 has an AC Foremost and a Tc-*LrW* gwm443 allele, but is susceptible (*lrW/lrW)*, therefore one chromosome is recombinant between these two loci and one is not. Two individuals (not shown in Figure 7) had two recombinant chromosomes. If resistant individuals were used, heterozygous individuals complicate mapping, as heterozygotes cannot be distinguished from homozygous progeny phenotypically on F2's, and requires a progeny test of F_3 families to be conducted, although mapping software is able to handle F_2 data.

Analyzing the F_2 population segregating for *LrW2* found no linkage between any microsatellite markers found to be polymorphic on chromosome 1D in this cross and the gene. This is likely due to the lack of useful polymorphic markers with simple electrophoretic patterns in this cross on the short arm of chromosome 1D. As individual 2-360 (Table 6 and Figure 10) inherited the Tc-*LrW2* gwm642 allele (1DL), the terminal half of the long arm can be eliminated as a candidate region for *LrW2*. Since a sufficient number of microsatellite markers were tested on the long arm of chromosome 1D and near the centromere showed no association to *LrW2* in the F_2 population, it is possible that *LrW2* is on the short arm of chromosome 1D distal of gwm337. Four genes/alleles, *Lr21*, *Lr39*, *Lr40* (Huang and Gill, 2001), and *Lr42* (Cox *et al.* 1994), have all been reported on chromosome 1DS, and *Lr41* is somewhere on chromosome 1D (Cox *et al.*

1994), therefore it is currently unknown if *LrW2* is a unique gene, or a unique allele of a previously described gene, or the same gene or allele that has been previously described, but found from a different source. *Lr21, Lr39, Lr40, Lr41,* and *Lr42* were all found in *Aegilops squarrosa* (Cox *et al.* 1994; Huang and Gill, 2001) whereas *LrW2*, which was found in *Triticum aestivum* (Dyck, 1994).

Gene effectiveness

For both *LrW* and *LrW2* no *P. triticina* isolates were virulent at the seedling stage. The infection types found on Tc-*LrW* were almost always lower than Tc-*LrW2*. This is similar to how these two genes perform in the field, where *LrW* offers stronger resistance than *LrW2* (Brent McCallum, personal communication). Dyck and Jedel (1989) found that none of the nine *P. triticina* races tested were virulent on *LrW*. Dyck (1994) challenged *LrW2* with ten different races, of which one race, TBB, was virulent. This study found no virulence from the 29 isolates used, although TBB was not used here.

On several occasions it was noticed that *LrW2* was difficult to detect at the seedling stage when it was heterozygous. Detection improved in heterozygotes at more advanced plant stages, but the infection was greater than on homozygous plants. This leads to the conclusion that *LrW2* is incompletely dominant and may function partially as an adult plant gene. This incomplete dominance has been observed with several other *Lr* genes (Kolmer and Dyck, 1994). This is a point to consider if seedling rust tests are being used in breeding programs wishing to select *LrW2*. In contrast, *LrW* showed complete dominance in hybrids, which were heterozygous for *LrW*, with infection types similar or identical to plants homozygous for *LrW*.

Although these genes are effective, especially *LrW*, it is important to use new sources of resistance responsibly. The durability of a resistance gene increases when it is combined with other resistance genes (Dyck and Kerber, 1985). Since finding new sources of resistance requires a great deal of resources it is important to manage new genes/alleles properly.

Temperature sensitivity

Expression of both *LrW* and *LrW2* were affected by temperature (Table 9). Leaf rust resistance genes have been shown to have a range of reactions depending on the incubation temperature. For example, *Lr17*, *Lr16*, and all alleles of *Lr3* have lower infection types as temperature increases (Dyck and Johnson, 1983). Other genes, such as *Lr18*, and *Lr30*, have higher infection types as temperature increases (Dyck and Johnson, 1983; Statler and Christianson, 1993). Finally, some genes are unaffected by temperature, including *Lr2a*, and *Lr19* (Dyck and Johnson, 1983).

Both *LrW* and *LrW2* reacted slightly different to temperature. Like *Lr18* and *Lr30*, *LrW2* had low infection types at low temperatures (≤ 18°C), and as temperature increased infection type increased (Table 9). However, *LrW* had the lowest infection type at 18°C, low/intermediate reactions at both 15°C and 21°C, and higher intermediate reactions at 24°C and 30°C (Table 9). Thus, there appears to be an optimum temperature low infection types in seedlings carrying *LrW*.

Although the effect of temperature on infection types for *Lr* genes is interesting for gene characterization, it seems that this information is not useful for predicting the field performance of a given *Lr* gene because the outdoor temperature is usually variable.

Although, if critical temperatures for gene effectiveness can be established, the suitability of an *Lr* gene for a given climatic region could perhaps be predicted. The data in a study by Statler and Christianson (1993), in which variable temperatures were also tested, show infection type can depend on exposure, at least in part, to this critical temperature. The most practical use of information found from temperature sensitivity studies is that plants can be grown at the proper temperature to select a particular *Lr* gene in the greenhouse or growth cabinet when inoculating with leaf rust.

Haploid deficiency mapping

This study reports the first time that phenotype has been used to detect the absence of the gene of interest in haploid deficiency mapping. In the first report of this technique, allele specific molecular markers were used to determine the presence of the two genes being studied (Thomas *et al.* 2001). Determining which individuals have failed to receive the critical chromosome using phenotypic data has its pitfalls. This was most evident with *LrW2*, as heterozygotes gave inconclusive seedling reactions when inoculated with *P. triticina*. Thus, when considering implementing this technique it is important to first determine the best method to detect the absence of the locus in question. One benefit of using molecular markers is the lack of gene dose dependence (i.e. incomplete dominance). A second, and related, benefit is that recessive genes could be mapped if their absence could be determined with molecular markers. Although one drawback of using molecular markers is that a tightly linked marker must already exist, which is often not the case. It may seem redundant to use haploid deficiency mapping for genes that already have a marker, however allele specific markers developed from

dominant marker systems, like AFLP and RAPD, only mark allele presence, and say nothing of chromosome location.

Though untested, it seems possible that recessive genes could be assigned to chromosomes using haploid deficiency mapping in certain cases when gene absence is determined phenotypically. For this to work a) the gene of interest must be carried by the euploid pollinator rather than the haploid female, and b) the gene must be expressed in the hemizygous condition. The majority of hybrids in such a scenario would not exhibit the trait being studied, however if the haploid failed to transmit the critical chromosome, or chromosomal region, the recessive trait would be expressed due to the absence of the dominant alternate allele. Following the identification of deficient hybrids the same methods could be used to determine the critical chromosome. This is similar to the variation of monosomic analysis performed by Dyck and Kerber (1981), in which a line carrying *Lr30* (recessive *Lr* gene) was crossed with a series of monosomic lines. All of the F_1 progeny were susceptible to leaf rust except for the critical cross with mono 4A. The cross with mono 4A produced F_1 plants that were resistant because the dominant alternate allele was absent.

In general, haploid deficiency mapping seems to be an efficient method for assigning genes to chromosomes. While monosomic analysis is a proven method, haploid deficiency mapping is an option that should be considered and does have advantages. On occasion monosomic analysis gives ambiguous results because the critical cross (cross between gene carrier and line monosomic for the chromosome the gene is on) does not have segregation that deviates significantly from normal segregation (J. Thomas, personal communication). Singh *et al.* (2001) found two crosses, between a

leaf rust resistant line, and two different monosomic lines, to have distorted segregation, and further testing was needed to resolve which was the critical cross. With haploid deficiency mapping, only the few individuals with the aberrant phenotype, or in the case of Thomas *et al.* (2001) failed SCAR transmission, need to be analyzed. Furthermore, analysis of these individuals with microsatellite markers is a relatively rapid process.

If haploid deficiency mapping is to be implemented effectively it is imperative that more than one plant deficient for the gene is analyzed. In this study the first leaf rust susceptible plants identified in each population, 1-109, and 2-39, had multiple chromosomal deficiencies (Table 2), producing ambiguous results. However, if, for example, three individuals are analyzed and common deficiencies are found then definitive conclusions can be reached. Since the recovery of leaf rust susceptible hybrids, in this study, was approximately 1%, it is recommended that at least 300 to 400 hybrid seeds should be generated for analysis.

Homoeologous exchange

When looking at the deficient chromosomes in susceptible hybrids (Tc-*LrW* [haploid]/AC Foremost [Figure 6] and Tc-*LrW2* [haploid]/AC Foremost [Figure 10]), there are often microsatellite markers that are transmitted from an otherwise missing chromosome. This was evident with microsatellite markers gdm126 on chromosome 1D in plants 2-39 and 2-360, gwm642 in plant 2-360 in the Tc-*LrW2* [haploid]/AC Foremost cross (Table 6). This was also apparent with microsatellite marker gwm497 on chromosome 5B in plants 1-109, 178, 1-280, 1-369, and 1-438 in the Tc-*LrW*

(haploid)/AC Foremost cross (Table 4). This might be due to recombination between homoeologous chromosomes during meiosis in the haploid parent.

Previous work has suggested that exchange can occur between non-homologous chromosomes, particularly chromosomes with relatively high degrees of similarity such as homoeologous chromosomes. When Sears (1939) produced hybrids by pollinating haploid wheat (n = 21) with euploid wheat (2n = 42), progeny with reciprocal translocations were recovered based on the chromosome pairing data. It has been well documented that bivalents are found during meiosis in haploid wheat (Gaines and Aase, 1926; Person, 1955; Riley and Chapman, 1957; Kimber and Riley, 1963; Jauhar *et al.* 1991). The findings of Jauhar *et al.* (1991) in wheat, and Jauhar *et al.* (1999) in durum wheat show that bivalents in haploids are predominantly intergenomic, presumably between homoeologous chromosomes. It has been demonstrated that in wheat each chiasma is associated with a single crossover event (Fu and Sears, 1973). Thus, it is entirely logical that the reciprocal translocations observed by Sears (1939) were a result of homoeologous recombination during the meiosis of haploids. Similar observations have been made in *Sorghum vulgare* L., where bivalents were found during meiosis of haploid plants (Endrizzi and Morgan, 1955; Reddi, 1968), and hybrids produced by pollination with diploid plants sometimes have reciprocal translocations (Endrizzi and Morgan, 1955).

Sears (1939) outlines four different models (Figure 3) of how gametes formed in haploid wheat can produce aneuploid hybrids when pollinated by euploid wheat. The first model proposed (Figure 3A) is the only scenario that allows for recombination to occur, however there would not be any recovery of reciprocal translocations, as the

chromosomes involved in pairing move to opposite poles. Therefore a combination of the four models is likely to occur in cases where reciprocal translocations are found. Leaf rust susceptible chromosome deficient hybrids found in this study conform to the first model (Figure 3A). This can explain the transmission of a small piece of an otherwise deficient chromosome from the haploid parent, which is shown more clearly in Figure 11. Note that deficiencies from recombination in the chromosome that paired with the critical chromosome are not detected with microsatellite markers because this chromosome is likely duplicated, as sister chromatids did not disjoin.

If reciprocal translocations were present in a hybrid in this study they would go undetected, as these hybrids would 1) carry the *Lr* gene in question, and 2) transmit the expected microsatellite marker alleles. The microsatellite markers would not show any abnormalities unless these individuals with reciprocal translocations were self-pollinated and microsatellite linkage relationships were studied. These events are of no interest in this study, as they do not shed light on the location of these genes in an easily detectable fashion.

In a similar study, Thomas *et al.* (2001) also found incomplete chromosome deficiencies. The results of this study (Figure 6 and Figure 10), and that of Thomas *et al.* (2001) support the first model outlined by Sears (1939, Figure 3A), that chromosome deficiencies from the haploid parent are the result of pairing between homoeologous chromosomes. Although it should be noted that haploids of some cultivars produce viable female gametes that are predominantly genetically normal (n = 21, ABD; Julian Thomas, personal communication), thereby producing hybrids of normal chromosomal constitution (2n = 3x = 42, AABBDD). This could be caused by genotypic factors

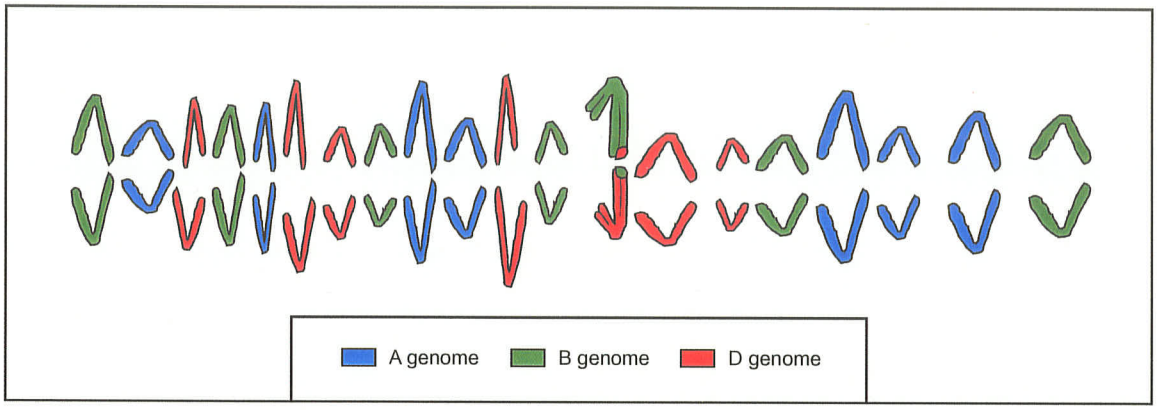

Figure 11 - Model of chromosome behaviour in early anaphase I during meiosis in haploid wheat. One bivalent has paired, and homoeologous exchange has occurred. The rest of the chromosomes undergo sister chromatid disjunction. If second division fails, two restitution gametes would form, each having a duplication and deficiency. The deficiency is incomplete because of the transmission of the recombinant chromatid in the bivalent. Determining which chromosome was involved in the recombination using microsatellites is not possible because of the duplication.

controlling homoeologous pairing, thus limiting aneuploid gametes in differing genetic backgrounds. This seems unlikely, as a wide range of studies have shown the average bivalent frequency in haploid wheat is approximately one (Person, 1955; Riley and Chapman, 1957; Kimber and Riley, 1963; Jauhar *et al.* 1991). An alternative hypothesis may be that genotypic factors in some cultivars result in the failure to support gametes with abnormal chromosomal constitution. In any case cultivars whose haploids do not produce viable chromosome deficient gametes are not suitable for use with the haploid deficiency mapping technique. This is difficult to predict and perhaps trial and error is the only approach for determining cultivar suitability until this phenomenon is understood.

The differences in the variability of the transmission of critical chromosomal segments through the haploid parent between Tc-*LrW* and Tc-*LrW2* are interesting, although the sample size is relatively small. All of the susceptible hybrids from the Tc-*LrW* (haploid)/AC Foremost population were deficient for the same microsatellite markers on chromosome 5B, but inherited the Tc-*LrW* allele of gwm497 (Table 4 and Figure 6). The chromosome deficiencies in these cases could be considered consistent. In contrast, the four susceptible hybrids found in the Tc-*LrW2* (haploid)/AC Foremost population had three different distinguishable chromosome 1D deficiencies. One plant, 2-39, inherited the Tc-*LrW2* allele of gdm126. Another plant, 2-360, inherited the Tc-*LrW2* alleles of gdm126 and gwm642. Two plants, 2-271 and 2-397, had no detectable transmission of Tc-*LrW2* 1D chromosomal segments (Table 6 and Figure 10). A possible explanation of why these differences have been observed could be differences in homology between homoeologous chromosomes. It was been shown that the A and D

genomes of wheat have a higher degree similarity than the B genome has with the A and D genomes (Jauhar *et al.* 1991). Also, chiasmata occur between chromosomal segments that have homology, even if the chromosomes in question are non-homologous (Person, 1955; Riley and Chapman, 1957). Since crossing over is a function of homology there could be "hot spots" of recombination between homoeologous chromosomes during haploid meiosis. If two chromosomes have more homology between them it is conceivable that chiasma could form over a greater length of the chromosome. However, if homology, at the level required for crossing over, is restricted to a relatively small region crossing over may be limited to this region, which may explain crossing over "hot spots" (Figure 12).

As the B genome seems to differ the most from the A and D genomes (Jauhar *et al.* 1991), it could be that only a small conserved region of chromosome 5B can cross over with homoeologues from the A and B genomes, resulting in the observed consistent transmission of the Tc-*LrW* gwm497 allele. Conversely, the variation in Tc-*LrW2* chromosome 1D transmission could be due to higher homology with the 1A, resulting in recombination over larger regions (Figure 12). Recombination between chromosomes 1D and 1B would presumably be restricted in terms of site of crossing over. However, if recombinant products from 1D/1A and 1D/1B are both found in population, it is conceivable that an array of products would still be found, mostly due to the variable recombinant products between chromosomes 1D and 1A.

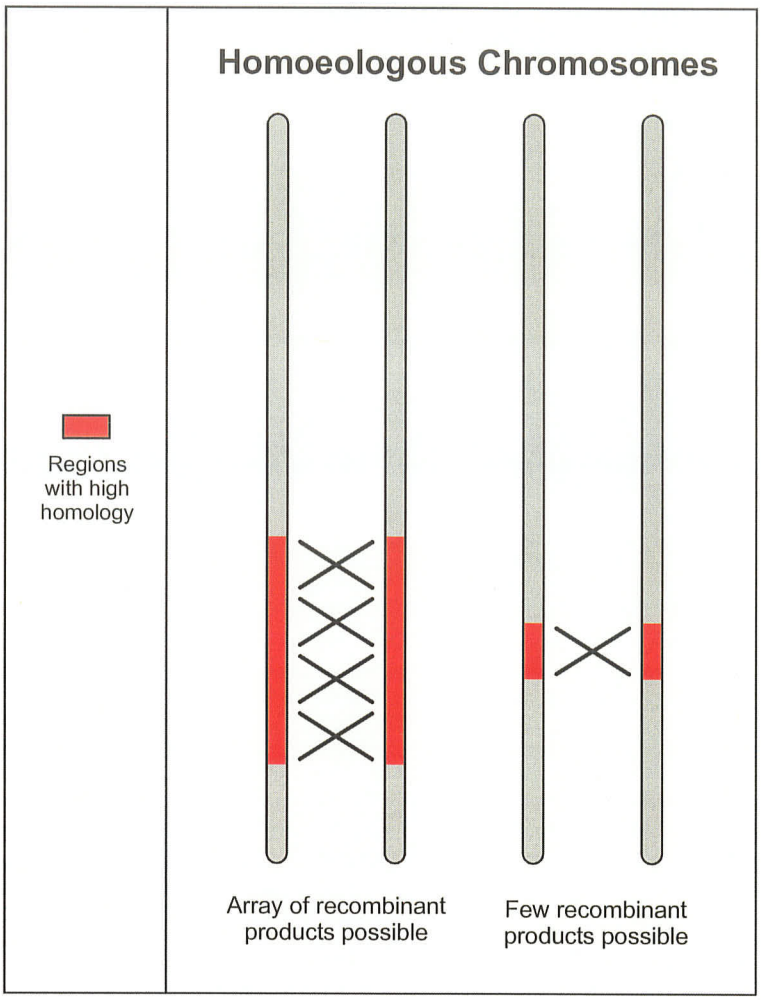

Figure 12 - Representation of two pairs of homoeologous chromosomes and their ability to recombine. Recombination is a function of homology, therefore chromosomes with larger regions of high homology (eg. homoeologues from the A and D genomes) can potentially produce many different recombinant products because crossing over could occur at any location within a larger region (left). If the region where homology is great enough for crossing over to occur is relatively small (right) (eg. homoeologues from the A and B, or B and D genomes), then a limited number of different recombinant products is possible.

Further Research

Three point linkage with LrW

In order to definitively place *LrW* on a chromosome 5B map interval three-point linkage analysis needs to be done. The nearest polymorphic proximal microsatellite is wmc149 (Table 5, no linkage to *LrW*), which is reported to be 36 cM from gwm443 (Figure 6). If gwm443 and wmc149 show linkage in this cross, then with the addition of *LrW* a three-point linkage map of the distal portion of chromosome 5BS could be constructed. This would accomplish two things, 1) give a more precise description of the location of *LrW*, and 2) provide a more concrete starting point for any high-density mapping and cloning projects.

Additional mapping with LrW2

It is highly likely that *LrW2* is on chromosome 1D, as five leaf rust susceptible hybrids from the array of aneuploids all are missing portions of chromosome 1D from Tc-*LrW2* provides strong evidence. However additional data is needed to confirm the location of *LrW2*, and determine its uniqueness from other leaf rust resistance genes on chromosome 1D needs to be determined. To test the uniqueness of *LrW2* it is necessary to conduct allelism tests with *Lr* genes that are also on chromosome 1D. As the uniqueness of *Lr39*, and *Lr40* are unclear and appears to be allelic with *Lr21* (McIntosh *et al.* 1995; Huang and Gill, 2001), it is necessary to only test *Lr21*, *Lr41*, and *Lr42*.

A population was generated by crossing a leaf rust resistant male parent (carrying *LrW2*), which was monosomic for chromosome 1D, with a euploid (2n = 6x = 42) line that was leaf rust susceptible. This cross was made in anticipation of performing

monosomic analysis to confirm our findings that *LrW2* is on chromosome 1D. However, the incomplete dominance of *LrW2* did not allow for accurate phenotyping at the seedling stage, thus observing distorted segregation is unreliable. This population is still useful, because individuals that have 41 chromosomes should not produce any resistant progeny when allowed to self-pollinate, and all individuals with 42 chromosomes should produce resistant progeny when allowed to self-pollinate. This would definitely confirm that *LrW2* is on chromosome 1D.

Tightly linked markers to LrW *and* LrW2

For marker-assisted breeding it is important to have markers that are tightly linked to the gene being selected to avoid high frequencies of false positives in a given population. Both *LrW* and *LrW2* are promising genes for wheat breeders, therefore developing closely linked markers would be of interest. The estimated genetic distance between gwm443 and *LrW* of 14.1cM is too large to be highly useful because recombination between the gene and marker would occur at a frequency unsatisfactory for marker assisted selection. So far no markers have shown any linkage to *LrW2*. Perhaps it will be necessary to use techniques such as RAPD, or more likely AFLP, to produce easy to use SCAR markers. If such markers were developed pyramiding of these *Lr* genes in a breeding program would become possible.

6. Conclusion

It was shown with haploid deficiency mapping that *LrW* is on chromosome 5B. Analysis of an F_2 population discovered that the *LrW* locus is 14.1 cM from the microsatellite marker gwm443, on the short arm of chromosome 5B. This gene is effective against *P. triticina* populations in North America, and is a promising resistance source for Canadian wheat breeding programs.

Results from haploid deficiency mapping place *LrW2* on chromosome 1D. No linkage with microsatellite markers was found, but evidence suggests that *LrW2* could be on the short arm of chromosome 1D. Although not as effective as *LrW, LrW2* holds promise for wheat breeders. The uniqueness and relationship of *LrW2* to other *Lr* genes on chromosome 1D requires further investigation.

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