ABSTRACT

FLEMMIG, EMMA LEIGH. Molecular Markers to Deploy and Characterize Stem Rust Resistance in Wheat. (Under the direction of Dr. Gina Brown-Guedira and Dr. David Marshall).

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a wheat (*Triticum aestivum* L.) pathogen of global importance with the potential for devastating yield losses. *Sr2* is a race non-specific stem rust resistance gene that has remained effective for over 80 years. *Sr2* does not offer complete resistance to any races of stem rust, but its slow-rusting phenotype offers protection when major effect *Sr* genes breakdown. *Sr2* is an excellent candidate for marker-assisted selection (MAS) and gene pyramiding since it has a very predictive DNA marker available. Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* in the United States, is a serious threat to human health and wheat quality in parts of the United States. Management of FHB is difficult with fungicides, and host resistance is quantitative. The FHB resistance QTL of largest effect is *Fhb1*. *Fhb1* also is an excellent candidate for MAS and gene pyramiding, as there are very accurate markers for *Fhb1*. *Sr2* and *Fhb1* are not found together in cultivars because they are in repulsion linkage on chromosome 3BS. With the assistance of DNA markers, recombination was detected between *Sr2* and *Fhb1* to couple the genes in a soft red winter wheat (SRWW) population. Efforts continue to deploy *Sr2-Fhb1* as a unit in SRWW germplasm.

During attempts to break repulsion linkage between stem rust resistance genes *Sr36* and *Sr40* on chromosome 2BS, we discovered that *Sr36* has very strong segregation

distortion. We were not able to recover *Sr36-Sr40* recombinant plants, but our interest in the *Sr36 Triticum timopheevii* introgression on 2BS was piqued. We utilized data from 367

SRWW cultivars and breeding lines from the eastern U.S. with dense single nucleotide polymorphism (SNP) marker coverage to determine the size of the *T. timopheevii* introgression containing *Sr36.* The 576 markers allowed us to create a visualization of the size of the 2G introgression into chromosome 2B. We determined that there are two different sources of the *T. timopheevii* segment in eastern winter wheat. When either introgression is present in lines, allelic diversity is greatly reduced along the length of chromosome 2B. Both introgressions are associated with a high 1,000 grain weight allele, while the two introgressions are linked to differing photoperiod alleles on 2B. Our new information on the introgressions and other 2B alleles can provide insight to breeders about whether to use *Sr36* in their programs and which introgression they will want to choose from its affiliated traits.

Molecular Markers to Deploy and Characterize Stem Rust Resistance in Wheat

by
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DEDICATION

To the best parents in the world.

BIOGRAPHY

Emma Flemmig was raised in Glidden, Iowa, by her parents Edward Flemmig and Jennifer Fairchild-Flemmig. After pursuing agricultural interests in high school, she decided to attend Iowa State University. In 2010, she received her Bachelor of Science in Agronomy and International Agriculture. Promptly after finishing her degree, she moved to Raleigh, North Carolina, to work with Dr. Gina Brown-Guedira and Dr. David Marshall on stem rust projects in the Eastern Regional Small Grains Genotyping laboratory. After completion of her Master of Science at North Carolina State University in Crop Science, Emma will move to Blacksburg, Virginia. There she will begin a Ph.D. program with Dr. Wade Thomason in Crop & Soil Environmental Sciences at Virginia Polytechnic Institute and State University.

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A million thanks to Gina.

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Global Significance

Bread wheat (2n=6x=42), *Triticum aestivum*, is one of the most important crops in the world. Wheat is the world's third largest grain crop and is cultivated on the largest land area (FAO, 2012a). Wheat is the greatest source of carbohydrates in temperate climates (Leonard & Martín, 1963) and is higher in protein than all the other major cereal crops (USDA ARS, 2011). More metric tons of wheat are traded annually than any other cereal (FAO, 2012a). The economic, environmental, and health aspects of wheat production and availability impact everyday life for individuals all over the world.

Modern bread wheat is most productive in temperate climates, 30° – 60°N and 27° – 40°S, but is grown far beyond these limits (Curtis, 2002). Three-fourths of the world's wheat is grown in moderate rainfall conditions between 375 and 875 mm, but it can also be grown very far outside this range if adapted to arid or tropical climates (Leonard and Martín, 1963). Winter wheat is planted in the fall, and heading is delayed until after the 0°-5°C season. Spring wheat is planted in the spring, flowers in the heat, and is harvested before the cold season (Curtis, 2002). Wheat is grown over the widest ranges of precipitation and latitude of the world's major crops.

Wheat Production in the United States

American farmers harvest 45 to 55 million acres of wheat annually (USDA, 2012). This acreage produces around two billion bushels of wheat worth about $15 billion (USDA,

2012). There are six market classes of wheat in the U.S., including hard red winter, hard red spring, soft red winter, hard white, soft white, and durum. These classes tend to be grown regionally in the U.S. and have different end uses (Bonjean & Angus, 2001).

Hard red winter wheat (HRWW) is the dominant wheat class in the U.S. It is grown in the wheat basket of the lower Great Plains, it is the most exported market class. Variable in protein and gluten, but higher than soft red winter wheat, HRWW is very useful for milling and baking. It is the predominant wheat for bread and roll production and all-purpose flour. States producing mostly HRWW are South Dakota, Nebraska, Kansas, Oklahoma, Texas, Colorado, New Mexico, and Wyoming. North Dakota and Montana produce some hard red winter wheat, put they produce more hard red spring wheat because of their harsher winters (Carver et al., 2001). Hard red spring wheat (HRSW) is produced in Montana, North and South Dakota, and Minnesota. HRSW has the highest protein content and is the best for milling and baking quality breads. It is also a highly exported market class (Busch & Rauch, 2001).

Durum wheat has the hardest grain of all wheat classes. It is used to make semolina flour for pasta production. It is the only tetraploid wheat (*Triticum turgidum* ssp. *durum*) grown on a commercial scale in the United States (Briggle & Curtis, 1987). Most of durum wheat production is in North Dakota. The rest of the production is in the other HRSW states, as well, as California and Arizona. Durum wheat has very high protein and moderately low gluten contents.

Soft white wheat is grown in two areas of the U.S. The first is at the northern ends of the Eastern winter wheat region in the states of New York and Michigan. The second region is the Pacific Northwest in Idaho, Washington and Oregon on the western side of the Rocky Mountains (Bacon, 2001; Peterson et al., 2001). Hard white wheat is grown in the same region of HRWW, primarily Kansas. Less breeding has been done for the hard white wheat market class as it the newest class. Hard white wheat has a milder taste but similar properties to HRWW. It is used for hard rolls, yeast breads, tortillas, and oriental noodles (Carver et al., 2001). Soft white wheat is lower in gluten and protein than hard white and is used primarily in breakfast cereals and other similar products as soft red winter wheat. (Bacon, 2001). Both classes of white wheat are more susceptible to pre-harvest sprouting than red wheats (Flintham, 2000).

Soft red winter wheat (SRWW) is the dominant wheat market class of the Eastern and Atlantic U.S. It is a large region and represents more diverse climates and topography than all other U.S. wheat market classes. States primarily growing SRWW are Louisiana, Georgia, South and North Carolina, Virginia, Maryland, Pennsylvania, Arkansas, Missouri, Illinois, Indiana, Tennessee, Kentucky, Ohio, and Michigan (Bacon, 2001). The SRWW production area follows the southern Mississippi River up the Ohio River towards the Great Lakes Region, and on the eastern side of the Appalachians from the Florida border to New York. The one common climate condition these two regions have is humidity, so SRWW regions tend to have higher fungal disease incidence than the hard wheat regions. The lower protein and gluten content of SRWW makes it useful for many baked goods – biscuits,

cookies, cakes, crackers, yeast breads – except for traditional breads and hard rolls (Bacon, 2001). In 2011, production of SRWW was around 450 million bushels in the U.S. (USDA, 2012).

Wheat Evolution

Origin of Bread Wheat

Wheat played an important role as the staple crop in the Near East origin of agriculture, and thus the foundation of Near Eastern and Western civilization. Beginning in Mesopotamia, in settlements between the Euphrates and Tigris Rivers, wheat – along with barley, rye, and alfalfa – was domesticated near the present day borders of Iraq, Iran, and Turkey around 11-12,000 years ago (Harlan, 1992, Huen et al., 1997). Wild einkorn, emmer wheat, and barley are found along the Fertile Crescent, supporting the origin of wheat and barley domestication in Iran (Harlan, 1992). Wheat provided a food that could be stored significantly longer than the traditional wares of hunting and gathering. As the first agrarian societies grew out of the Near East and into northern Africa and Europe, wheat seed was taken to these new locations and production expanded. Wheat moved through the East to China and India (Harlan, 1992). It was taken to the Americas and Australia by European settlers. Eventually wheat production moved to some of the most remote corners of the world.

Over time though, wheat changed. Farmers in different areas and their climates selected the best wheat plants to save as seed for planting the following year. By natural and

artificial selection in diverse environmental conditions, landraces of all different morphologies, nutritional content, abiotic and biotic stress tolerance, vernalization requirements and photoperiod responses were developed and used in different regions (Worland, 2001). This diversity is the basis on which wheat breeders around the world base their programs in research and cultivar development. Much of the world's wheat diversity has probably become extinct since the introduction of industrial agriculture, but the diversity that we managed to collect is housed in international and national germplasm banks (Fowler and Mooney, 1990). Wheat breeders and scientists use these banks to continue to introduce qualities from exotic germplasm in their breeding programs to combat changes in climate, pests, and disease pressures.

Wheat Domestication and Wild Relatives

Bread or common wheat (*Triticum aestivum*) is in the Poaceae family with the other cereal crops and grass species. Though very similar to the small grains, wheat and its closest wild relatives are the only species in the Triticeae genus. *T. aestivum* is an allohexaploid (BBAADD genomes, 2n =6x =42) and was domesticated from two hybridization events of three grasses with seven pairs of chromosomes each. Wild einkorn wheat (*T. monococcum* ssp. *aegilopoides*), a diploid (A^MA^M), was domesticated in the Near East, generating the very similar, cultivated einkorn wheat (*T. monococcum* ssp. *monococcum*, also A^MA^M) (Feldman, 2001; Harlan, 1992; Huen et al., 1997). Cultivated einkorn (2n=2x=14) is a hulled wheat with a non-brittle rachis.

It is believed that tetraploid wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), having BBAA genomes, was generated by a hybridization of *Triticum urartu* (AA genome) and *Aegilops speltoides* (SS genome) or another potentially extinct *Aegilops* (S^XS^X genome) species in Turkey (Curtis, 2002; Orzan et al., 2002). Domesticated from wild emmer, the first cultivated tetraploid wheats (BBAA, 2n=4x=28) were Georgian wheat (*T. turgidum* ssp. *paleocolchicum*) and cultivated emmer (*T. turgidum* ssp. *dicoccon*), both hulled species. Many free-threshing tetraploid wheats (other ssp. of *T. turgidum*) were developed from Georgian and emmer wheats (Feldman, 2001). Among the tetraploid wheats, only durums (*T. turgidum* ssp. *durum*) are cultivated on any scale today (Bonjean & Angus, 2001).

Another important tetraploid wheat that has been used for improvement of modern bread wheat is cultivated *T. timopheevii* ssp. *timopheevii* (GGA^tA^t). Cultivated timopheevii has a very narrow geographic distribution—primarily restricted around the country of Georgia (Badaeva et al., 1994). Wild timopheevii (*T. timopheevii* ssp. *armeniacum*) was generated from a hybridization of *T. urartu* and *Ae. speltoides*. Unlike domesticated timopheevii, wild timopheevi has a wide geographic distribution in the Middle East and surrounding countries and, most likely, originated in Iraq (Badaeva et al., 1994). Wild einkorn wheat is believed to have hybridized with cultivated timopheevii to generate a hulled hexaploid species *T. zhukovskyi* ($GGAAA^MA^M$) (Feldman, 2002).

The earliest hexaploid wheats (BBAADD, 2n=6x=42) (*T. aestivum* ssp. *spelta* (spelt) and ssp. *macha*) were most likely generated from a hybridization event of *Aegilops*

tauschii (DD) and a wild or cultivated tetraploid wheat species (BBAA) in Turkey (Feldman, 2001; Orzan et al. 2002). Free-threshing bread (ssp. *aestivum*), club (ssp. *compactum*), and Indian dwarf wheats (ssp. *sphaerococcum*) were generated from selections of mutants of spelt wheat (Feldman, 2002). While the diploid *Aegilops* and *Triticum* species were great sources of genetic diversity, each hybridization event into cultivated tetraploid and hexaploid wheats was a great genetic bottleneck. These wild ancestors hold great potential as sources of new, useful genes for wheat breeding.

Stem Rust

Historical Impact

Stem rust (*Puccinia graminis*), also known as black rust, is one of the oldest documented and most devastating pathogens of cereal crops. Documented since biblical times, stem rust has existed as nearly as long as wheat cultivation (Peterson, 2001). Its early ancestors are thought to have originated on the Berberidaceae family in central Asia, eventually transferring the telial stage to grass hosts and maintaining the aecial cycle on barberry (Leppik, 1959). Stem rust spread from Asia through the Middle East to Europe and Africa, and eventually to the Americas and Australia (Leonard & Szabo, 2005).

A seemingly healthy crop can suddenly be lost to an explosion of stem rust only a few weeks before harvest—a pattern that has devastated wheat farmers for centuries. The ancient Romans, Greeks, and philosophers of the Middle Ages all made written records documenting the presence of stem rust epidemics (Peterson, 2001). Whereever wheat went,

stem rust followed. Stem rust is not only a part of ancient agriculture but continues to threaten wheat production into the modern history of the United States. The early 1900s were particularly important in modern history of the American battle with stem rust. In 1904, the upper Midwest suffered losses of about $10 million. Then in 1916, the same states suffered an even larger epidemic with losses of $181 million. These epidemics inspired a team of American pathologists to champion the eradication of barberry during World War I. The program was very successful—there was a decrease in the number of overwintering *Pgt* races from 20 to five (Campbell and Long, 2001). Barberry eradication reduced sexual recombination and overwintering inoculum in the northern wheat states. Since the winters are too cold in the northern U.S. for mycelium to overwinter, stem rust needed to reestablish itself every spring from urediniospores blowing north up the "*Puccinia* pathway" (Stakman, 1934).

Stem rust can attack all of the aboveground plant tissues including stems, leaves and glumes. Once infection is established, hyphae alter the direction of phloem transport and divert nutrients to the infected tissue. The sugars that accumulate support increased fungal growth and sporulation. Wheat plants infected with stem rust have reduced tillering, and fewer, shriveled seeds of low milling quality. Severe infection can also cause lodging of the wheat plants and increased infections from other pathogens (Leonard & Szabo, 2005). Controlling stem rust with fungicides is often not cost-effective for wheat producers. If a severe outbreak of stem rust were to occur in the U.S., hundreds of millions of tons of wheat could be lost to the disease (Agrios, 2005).

Life Cycle

There are five *formae speciales* of stem rust, each named for its most recognized host. *Puccinia graminis* f. sp. *tritici* (*Pgt*) is the causal agent of wheat stem rust. *Pgt*'s telial host is wheat and aecial host is common barberry, *Berberis vulgaris* (Jin, 2011). *Puccinia graminis* has a wide host range of the small grains and other grasses. It is a heterocious, macrocyclic rust fungus with five distinct spore stages of a basidiomycete. Stem rust is found in all wheat growing regions of the world (Agrios, 2005). It is most common in warmer regions (18-30°C) and flourishes when nighttime dews are followed by sunny days. Stem rust is historically a serious problem in the Great Plains of the U.S. Stem rust mycelium can over-winter in fall-infected wheat seedlings in the southern Great Plains, thereby serving as primary inoculum following sporulation when temperatures rise in the spring (Leonard, 2001).

The first symptom of stem rust infection is chlorotic flecks on the wheat stem, which become elongated blisters. Eventually, the orange-red, "rust-colored," pustules of urediniospores will erupt from the tissue, pushing the epidermis back (Leonard & Szabo, 2005). At the end of the growing season, telia form and produce teliospores. The dikaryotic, black teliospores overwinter on wheat straw. These teliospores give stem rust its other common name, black rust. The teliospores germinate in the spring with the alternate host barberry (*Berberis* and *Mahonia* spp.), undergo karyogamy and meiosis, to form four basidiospores. It was discovered in 1863 by the German scientist Anton deBary that stem rust was heteroecious and the alternate host was barberry (Campbell and Long, 2001).

The basidiospores germinate on the barberry species and form pycnia. There are two mating types of pycniospores—positive and negative—formed. When the positive and negative types come together an aecium is formed and aeciospores of unique haplotypes will infect wheat or other gramineous hosts. On wheat, uredinia form and generate many secondary cycles of urediniospore production though the growing season. The urediniospores rupture from the stems and other plant parts becoming the windborne inoculum for re-infection of wheat. This process allows for the transmission of stem rust on a potentially continental scale (Roelfs, 1985; Leonard & Szabo, 2005).

Stem Rust Resistance in Wheat

There are 52 named *Sr* genes, three of which have multiple resistance alleles (McIntosh et al., 1995; Singh et al., 2008; Faris et al., 2008; Bansal et al., 2009; Hiebert et al., 2010, 2011; Qi et al., 2011; Rouse et al., 2011; Liu et al., 2011; Qi et al., 2011). There are also a number of temporary designations for *Pgt* resistance loci where it is not clear whether they are new genes or new alleles (McIntosh et al., 1995; Hiebert et al., 2010, 2011). In total, more than 60 resistance alleles to stem rust have been identified, but not all are currently available in adapted germplasm. Some *Sr* genes are no longer effective, while others have undesirable agronomic traits linked to their introgression. Recently researchers have also found new, uncharacterized stem rust resistance genes in *T. monococcum* and *T. urartu* which have the potential to become new, effective *Sr* genes for use in common wheat (Rouse & Jin, 2011). The vast majority of *Sr* genes which have been deployed on a large

scale are currently recognized by virulent races of *Pgt*, leaving the breeding community interested in finding more durable ways to deploy the remaining, effective *Sr* genes.

The first source of host plant resistance to stem rust in U.S. germplasm was introgressed into *Triticum aestivum* from *T. turgidum* ssp. *dicoccon,* 'Yaroslav' emmer, and ssp. *durum,* 'Iumillo' durum, by E.S. McFadden into the cultivars 'Hope' and H44-24 (McFadden, 1930). The source of resistance was identified as a single gene (Knott, 1968) and named *Sr2* (Ausemus et al., 1946). *Sr2* was mapped by monosomic analysis to chromosome 3BS (Hare and McIntosh, 1979, Spielmeyer et al., 2003). Though the genotypes were not widely used due to the negative agronomic traits associated with linkage drag from the emmer introgression, 'Hope' and H44-24, have been widely used in breeding programs (McIntosh et al., 1995; Kolmer, 2001). *Sr2* has a recessive inheritance (Ausemus et al., 1946) and provides only adult plant resistance (Knott, 1968). Both qualities make it difficult to phenotype, but *Sr2* is tightly linked to the trait pseudo-black chaff (PBC). In spite of strong environmental effects on its appearance, PBC has been used to effectively detect the presence of *Sr2* (Kota et al., 2006). *Sr2* is linked to microsatellite marker *Xgwm533* (Spielmeyer et al., 2003), but the cleaved amplified polymorphic sequence (CAPS) marker *XcsSr2* is currently most diagnostic for its presence (Mago et al., 2011a).

Since *Sr2* is the only cataloged source of non-race specific stem rust resistance (Singh et al., 2006), it is an important source of durable stem rust resistance when pyramided with other race-specific genes. All *Sr* genes are seedling and adult-plant effective

and race-specific except *Sr2* (Singh et al., 2008). The unique phenotype of cultivars with *Sr2* is slow-rusting (Sunderwirth and Roelfs, 1980). The structures of most *Sr* proteins and sequences of *Sr* genes are not characterized, but it is expected that *Sr2* is not like typical resistance genes that code for a nucleotide-binding site-leucine-rich repeat (NBS-LRR) domain (Lagudah, 2011).

Sr36 and *Sr40* are both are located on chromosome 2BS (Tsilo et al., 2008; Wu et al., 2009). *Sr36* is in adapted materials, but *Sr40* had limited use in breeding programs due to its poor agronomic performance (McIntosh et al., 1995). The stem rust resistance gene *Sr36* was introgressed from *T. timopheevii* ssp. *timopheevii* [GGA^tA^t] in SRWW lines: CI 12632 and CI 12633 (Allard and Shands, 1954), while *Sr40* was introgressed from *T. timopheevii* ssp. *armeniacum* [GGA^tA^t] (Dyck, 1992). Both *Sr36* and *Sr40* are major resistance genes with seedling and adult, race-specific resistance. The markers most closely linked to *Sr36* and *Sr40* are *Xwmc477* and *Xwmc344*, respectively (Tsilo et al., 2008; Wu et al., 2009).

Stem Rust Differentials

Sr genes have variable efficacy to different races of stem rust. A collection of named *Sr* genes, are used for classifying the race of stem rust. The original North American nomenclature system for distinguishing *Pgt* races was developed using only 16 unique *Sr* differentials (Roelfs & Martens, 1988). A differential is a cultivar that is believed to only contain one resistance gene; in this case, only one *Sr* gene. The letter is based on the low or

high infection type a *Pgt* strain has on the differentials. Utilizing a four-letter system, with four differentials per letter, means that there are up to 65,536 *Pgt* races that could be named. Most of family of Ug99 races would be classified as TTKS with the original 16 differentials, yet not all would have the same overall virulence profile, so a fifth set of four new differentials (one letter) was added (Jin et al., 2008b).

Emergence of the Ug99 Family

From 1955-2001, globally there were no stem rust epidemics with more than 5% yield losses (Peterson 2001). In Uganda during 1999, Pretorius et al. (2000) identified a race of *Pgt* that was virulent on wheat cultivars known to carry the 1RS:1BL translocation. The 1RS.1BL translocation is the short arm of rye chromosome 1R translocated on the long arm of wheat chromosome 1B and contains the *Sr31, Lr26*, *Yr9,* and *Pm6* genes, where the abbreviations are *Lr* for leaf rust resistance; *Yr*, stripe rust; and *Pm*, powdery mildew. This was the first appearance of *Sr31* virulence in *Pgt*, an *Sr* gene widely deployed around the world. Urediniospores of this 'Ug99' race were tested on differentials. Virulence was found to *Sr5, -6, -7b, -8a, -8b, -9b, -9e, -9g, -11, -15, -17, -21, -30, -31,* and *-38* (Pretorius et al., 2000; Wanyera et al., 2006, Jin et al., 2007). This was also the first virulence found to *Sr38*. TTKS was the first stem rust race to overcome every widely-used source of stem rust resistance in most international wheat breeding programs (Njau et al., 2010). Ug99 was immediately recognized as a new threat to wheat production and the warning was sounded around the world.

Ug99 was identified in Kenya and Ethiopia in 2005 (Jin et al., 2008a). Increased research, led by CIMMYT and USDA-ARS with the assistance of the Kenyan Agricultural Research Institute, on stem rust started in the mid-2000s. Now Ug99 is recognized by agriculturalists the world over, and its movement is closely monitored by wheat breeders and pathologists around the world and reported to the Food and Agricultural Organization of the United Nations (FAO, 2012b).

Ug99 was later named TTKS by the North American Stem Rust Nomenclature protocol (Roelfs and Martens, 1988; Wanyera et al., 2006), then TTKSK by the updated North American Stem Rust Nomenclature (Jin et al., 2008b). It was identified in Yemen and the Sudan in 2006 (Jin et al., 2008a; FAO, 2012). TTKSK's presence was confirmed in Iran in 2007 (Nazari et al., 2009), and in Tanzania in 2009 (FAO, 2012b). Fortunately, scientists have managed not to inadvertently transport spores to any of the world's major wheat regions like northern India and Pakistan, Australia, the E.U., or the U.S. In spite of good luck and conscientious travelers, Ug99's movement is following continental wind patterns. After Iran, it is expected to move more east and south towards India and Pakistan. There are also hypotheses that it may blow north through Turkey and into the E.U., from South Africa to Australia, and/or across the Atlantic to Brazil (Hodson, D. P., personal communication). Although it has moved slower than originally expected, breeders around the world are preparing for its arrival with improved resistance, using effective *Sr* genes.

Since 1999, TTKSK has not stopped evolving. The close monitoring of Ug99's spread outside of Eastern Africa allowed pathologists to track if its virulence is changing.

Relative strains have been found with additional virulence in Kenya: TTKST, with *Sr24* virulence (Jin et al., 2008b), and TTTSK, with *Sr36* virulence (Jin et al., 2009). Both of these strains have only been found in Kenya and Tanzania (FAO, 2012b). They are called the Ug99 family of races, because they form an evolutionary branch of *Pgt*, separate from older strains. It is important to note that a later analysis of TTKSK race found virulence on *Sr21* differentials (Wanyera et al., 2006). These and other descendents of TTKSK—TTKSF (*Sr31* avirulence), TTKSP (*Sr31* avirulence, *Sr24* virulence), PTKSK (*Sr31* virulence, *Sr21* avirulence), and PTKST (*Sr31* virulence, *Sr24* virulence, and *Sr21* avirulence)—are being monitored by the breeders and pathologists of national agencies throughout Africa, the Middle East, and India (FAO, 2012b). Some of these strains have been found as far south as South Africa and Zimbabwe in 2009 (FAO, 2012b). Table 1 lists the genes currently effective against TTKSK and their origin.

The Ug99 family of races are very divergent from *Pgt* isolates from any part of the world, including Africa (Jin et al., 2008b). The last major epidemic of stem rust was in the 1990s in Ethiopia (Singh et al., 2008). There is much speculation for the reason why the Ug99 family and *Sr31* virulence originated from Uganda and Kenya. The 1RS.1BL translocation carrying *Sr31* was widely deployed around the world. It is estimated by CIMMYT to have been in as much as 70% of wheat varieties (Singh et al., 2008). Respective to other regions, East Africa is a small player in the world of wheat production. Scientists have no definitive answer as to why Ug99 emerged there. The current hypothesis is that a combination of the inoculum load from the early 1990s, the presence of barberry in

the Kenyan and Ethiopian highlands, and the year round presence of living wheat tissue created the right environment for the TTKSK race to emerge in east Africa (Jin, Y., personal communication).

Fusarium Head Blight

Historical Impact

Fusarium head blight (FHB) is referred to as "scab" because of the sticky substance it produces, making the chaff adhere to the wheat kernel (Shaner, 2003). FHB was first described in the eastern U.S. in the 1890s. Severe epidemics plagued the U.S., Canada, Asia, and South America in the early 1900s. FHB was initially identified in China in 1936. It still seriously affects about a quarter of Chinese wheat production (Shaner, 2003). The disease has become a more widespread problem in North America since the 1980s, and there are reports of it throughout Europe in the later part of the 20th century (Parry et al., 1995).

FHB is a serious threat to wheat production and human and livestock health. Yield losses may be up to 50% (Agrios, 2005). It is particularly devastating because it can completely destroy a healthy crop only a few weeks before harvest (McMullen et al., 1997). The fungus biosynthesizes trichothecene toxins like vomitoxin (DON, deoxynivalenol). Vomitoxin inhibits protein biosynthesis in eukaryotes, so humans and animals can experience vomiting, diarrhea, and convulsions. Livestock will initially have reduced feeding, later start vomiting, and will eventually experience alimentary hemorrhaging.

Long-term exposure causes neurological problems and suppression of the immune system. Fungicide use to control FHB is limited by chemical costs. The tolerance levels for DON are only 1 ppm in grain destined for human consumption in the U.S. (FDA, 2010).

Life Cycle

The primary causal agent of FHB in North America is *Fusarium graminearum* [teleomorph *Gibberella zeae*]. FHB causes the most damage in the northern Midwest – the Dakotas, Minnesota, Illinois, Indiana and Ohio—in both HRSW and SRWW regions (Agrios 2005). *Fusarium graminearum* is a monocyclic ascomycete that overwinters as saprophytic mycelia on infected wheat, barley or maize stubble (Goswami and Kistler, 2004). In the spring, the optimum conidia germination temperature is 20-30°C, with more than 24 hours of moisture conditions. FHB is considered a warm, wet climate disease. In reality, it can grow on a wide range of temperatures. Conidia can even germinate just above freezing at 4°C for 48 hours (Shaner, 2003). The perithecia are the sexual structures that produce ascospores. They develop from the mycelia to be distributed in sync with the flowering of wheat heads. The first symptom of FHB is a watersoaked appearance on the spikelets. (Agrios, 2005).

Wheat is most susceptible to FHB during anthesis, or flowering. The latent period for infection is 3-11 days. The longer the wheat head is exposed to the FHB inoculum, the more severe the infection. The more days the wheat is exposed to moisture during anthesis, the risk of infection becomes greater. Post water-soaking appearance, FHB infection

appears as dark necrotic lesions on the glumes and eventually lightens to a bleached 'blight' appearance on the heads. The spreading from floret to floret is through the xylem and phloem in the rachis and rachilla, transitioning from a biotrophic to necrotrophic relationship quickly after infection (Goswami & Kistler, 2004).

FHB Resistance in Wheat

Many methods have been utilized to find resistance genes for FHB including populations of recombinant inbred lines (RILs), doubled haploid lines (DH), or near isogenic lines (NILs). All three of these methods are successful for detecting FHB quantitative trait loci (QTL) when utilizing populations large enough to detect marker linkage to a resistant phenotype. FHB resistance QTL have been found on all wheat chromosomes except 7D (Buerstmayr et al., 2009). Using restriction fragment length polymorphism (RFLP) markers in the earliest mapping population, the named QTL—*QFhs.ndsu-3B* on 3BS and *QFhs.ndsu-2A* on 2AL—and QTL on 6BS and 4B were identified in a population of RILs derived from the cross of 'Sumai 3' and 'Stoa,' a resistant and a moderately susceptible parent, respectively. *QFhs.ndsu-3B* was from 'Sumai 3' and *QFhs.ndsu-2A* was identified from 'Stoa' (Waldron et al., 1999). Amplified fragment length polymorphism (AFLP) markers and simple-sequence repeat (SSR) markers were used to map FHB QTL in a RIL population from the cross of resistant 'Ning 7840,' a Chinese cultivar derived from 'Sumai 3,' by susceptible 'Clark' in a second early study. One major QTL was found on 3BS, flanked by SSR markers *Xgwm533* and *Xgwm493* (Bai et al., 1999; Zhou et al., 2002).

In a third study, two populations—both utilizing Sumai 3-derived resistance—were used to map FHB QTL using a combination of RFLP, ALFP, and SSR markers. The first population was the same cross 'Sumai 3' by 'Stoa' from Waldron et al. (1999), and the second was 'ND2603' (Sumai 3/Wheaton) by 'Butte 85.' The 3BS and 6BS QTL of 'Sumai 3' were confirmed, two new QTL on 3AL and 6AS were identified in ND2603, and the 4BS and 2AL 'Stoa' QTL were confirmed (Anderson et al., 2001). Many more studies were conducted mapping FHB QTL. The QTL identified in these studies are listed in Table 2.

Fhb1

Three major QTL are repeatedly found in studies – *Fhb1* on 3BS, *Qfhs.ifa-5A* on 5AS, and *Fhb2* on 6BS (Buerstmayr et al., 2009). The useful resistance gene *Fhb1* accounts for 20-60% of variation in FHB infection in QTL studies (Wang and Miller, 1988; Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002, 2003; Zhou et al., 2002). Pumphrey et al. (2007) found that *Fhb1* reduced diseased kernels by 27% and FHB severity ratings by 23% in the field using a population of *Fhb1*-NILs. *Fhb1* is the only major resistance QTL with diagnostic markers (Zhou et al., 2002; Anderson et al., 2007; Buerstmayr et al., 2009). *Xgwm533* was the SSR marker initially linked to *Fhb1* (Buerstmayr et al., 2009). The alleles associated with FHB resistance are not common in North American wheat germplasm, making this marker useful for MAS in most populations. *Fhb1* was fine-mapped on chromosome 3BS in the interval between *Xgwm533* and *Xgwm493* (Cuthbert et al., 2006). SSR marker *Xumn10* (SNP: *Xsnp3BS-11*) is most diagnostic for *Fhb1* (Bernardo

et al., 2012). *Fhb1* has very slight yield drag reported with it in western germplasm (von der Ohe et al., 2009), but a more recent study of NILs reported no yield drag (Bernardo et al., 2011). Therein lies an opportunity for utilizing the most tightly linked markers from Bernardo et al., (2012) to reduce the chromosome segment from Chinese chromatin.

Resistance Breeding

Molecular Markers

RFLP markers were the first type of DNA markers to be developed (Lander and Botstein, 1989). RFLPs used restriction endonucleases to digest a DNA sample, and the pattern of DNA fragments in gel electrophoresis indicated a DNA "fingerprint" (Lander and Botstein, 1989). RFLPs are time consuming, labor intensive, and basically obsolete due to improved DNA sequencing methods (Lander & Botstein, 1989; Tanksley et al., 1989). AFLP markers are similar to RFLPs, but they selectively amplify segments of DNA from the restriction digestions (Vos et al., 1994; Powell et al., 1995).

Fortunately, more user-friendly markers are commonly available in wheat. Microsatellites, or SSRs, are short repeating sequences of 2-6 nucleotides. They are ubiquitious in wheat genomes, stable, and often co-dominant. Markers are developed as florescent oligonucleotides (primers) designed to the flanking regions around the repeats (Ziętkiewicz et al., 1994). After using the primers in polymerase chain reactions (PCR), the size of the amplified fragments can be distinguished by the varying copy number of the repeat with DNA sequencing of the PCR products (Ziętkiewicz et al., 1994). SSR markers

that are verified for tight-linkage to a gene of interest can be used for genotyping for the presence or absence of the gene. If an exact copy number (i.e. allele size) is linked to the gene's resistance allele, while different copy number(s) are not associated, those variable allele sizes can be utilized for genotyping for the resistance gene (Song et al., 2005). SSR markers are very common and useful because their co-dominance allows us to detect heterozygous genotypes. SSR markers are used for genotyping a great variety of resistance genes and other traits in wheat like: wheat rust resistance, FHB resistance, vernalization, gluten strength, kernel weight, etc. (Song et al., 2005; Tsilo et al., 2008; Buerstmayr et al., 2009).

Single nucleotide polymorphism (SNP) markers detect single base pair substitutions in DNA sequence (Gupta et al., 1999). When two alleles with differing phenotypes are distinguishable by a SNP, primers are developed that tag each allele with a different florescent label. Software has made it very simple to genotype large numbers of DNA samples amplified with a SNP marker. A read of the sample's florescence is taken at the end of PCR and the genotypes of samples can be clustered visually. Sequence-tagged site (STS) markers are identical to SNP markers except they are replicable segments of DNA at known map locations (Olson et al., 1989).

SNP chips have been developed that provide researchers with thousands of data points from a single DNA sample. SNP chips usually have thousands of miniscule wells or beads, each with a unique SNP oligonucleotide bound to the beads. It is the equivalent of running thousands of SNP markers in infinitesimally-small wells. SNP chip arrays provide

visual data of each sample's florescence, or allelic intensity, per marker (Staaf et al., 2008). This allows a researcher to obtain thousands of genome-wide data points for many samples in a format that costs cents per data point. SNP chip technology is now available in wheat, and has been available in other crop and animal species for years (Lipshutz et al., 1999).

Utilizing Wheat Relatives in Breeding

Introgressions

There are many examples of introgressions from wheat relatives, particularly for disease resistance (Allard & Shands, 1954; Dyck, 1992; Friebe et al., 1996; Faris et al., 2008; Singh et al., 2008). The three genomes of wheat and other relatives' genomes share significant homology, so translocations from other species into hexaploid wheat occur frequently (Knott, 1987; Maan, 1987). Many introgressions have been made from diploid, tetraploid, and hexaploid secondary species into bread wheat, like wild and domesticated *Triticum, Aegilops,* and *Secale* species (Sharma & Gill, 1983). However, methods have been developed to utilize bread wheat's tertiary gene pool that includes *Agropyron*, *Hordeum*, *Elymus*, and other species (Jiang et al., 1994). Many of these introgressions are an important source of disease resistance in bread wheat breeding programs. Two very important introgressions in wheat are the 1BL.1RS *Secale cereale* translocation with stem rust (*Sr31*), leaf rust (*Lr26*), and stripe rust (*Yr9*) resistance and the t2BS.2GS.2GL.2BL *Triticum timopheevii* ssp. *timopheevii* introgression of stem rust (*Sr36*) and powdery mildew (*Pm6*)

resistance (Allard and Shands, 1954, McIntosh, 1988; Jorgensen & Jensen, 1973; Singh et al., 2008).

Geneticists found it strange that if wheat relative introgressions occur regularly, then they were curious why there are not more chromosome pairing problems during meiosis and mitosis in hexaploid and tetraploid wheat species. Researchers found that the *Ph1* (*pairing homoeologous*) gene controls the homoeologous pairing of chromosomes in wheat. Homoeologous pairing is the process of chromosomes from similar genomes pairing during meiosis, like chromosomes 3B and 3D, versus homologous pairing, when chromosomes of the same genome, like 3B with 3B, pair during meiosis. The presence of *Ph1* on 5BL maintains the diploid pairing of the three wheat genomes by preventing homoeologous chromosomes from forming chiasmata (Okamoto, 1957; Riley and Chapman, 1958; Sears, 1976). After this discovery, lines with mutated *Ph1* were utilized to introgress wheat relative chromatin or to reduce the size of former wild introgressions (Koebner and Shepherd, 1986; Zhang et al., 2005).

Often these alien introgressions have linkage drag in adapted germplasm, so it is valuable to reduce the size of an introgression using molecular markers. Olson et al. (2010) used molecular markers to detect recombination in the *Triticum boeoticum* introgression carrying *Sr22* with *T. aestivum*. The smaller *T. boeoticum* segment with *Sr22* should have more favorable agronomic qualities when used in wheat cultivars. While some introgressions have negative linkage drag effects, there is some evidence pointing to the rye translocations improving wheat hardiness and disease resistance (Ehdaie et al., 2003).

R Genes

Breeding for host plant resistance is the most cost-effective way for producers to control plant pathogens, including wheat scab and wheat stem rust (Ruckenbauer et al., 2001; McIntosh et al., 1995). In the 1940s and 50s, H.H. Flor identified the gene-for-gene concept of host resistance in the flax rust (*Melampsora lini*) and flax (*Linum usitatissimum*) pathosystem (Flor, 1955). The gene-for-gene concept illustrates the inheritance of compatible resistance reactions of a host and the pathogen. The host has an *R* gene which expresses an R protein—an elicitor. The host's R protein, directly or indirectly, interacts with the Avr products—effectors—of the compatible pathogen *Avr* gene. New *Avr* genes are continually generated as existing *Avr* genes evolve and overcome the host's resistance, then the host compensates by developing a new *R* gene to override the *Avr* gene (Martin et al., 2003; Bent & Mackey, 2007). This process continues as the 'evolutionary arms race' between hosts and pathogens in nature. This paradigm has provided breeders with many resistance genes to work with from related species and landraces. Unfortunately, in agroecosystems the life of *R* genes is greatly reduced, so researchers are looking for ways to increase the lifespan of *R* genes in cultivars. R genes tend to be effective longer when pyramided with other *R* genes, when deployed as a mixture of single *R* gene cultivars in the field (multilines) or around a geographic region (Crute & Pink, 1996).

Quantitative Resistance

Quantitative (polygenic) resistance differs from qualitative resistance by providing resistance through multiple minor genes of smaller, incomplete effect. Often the effect of the minor genes or quantitative trait loci are indistinguishable from one another (Knott, 1988). This form of resistance in called horizontal resistance because it is non-race specific. Qualitative, or vertical, resistance uses one gene of major effect for race-specific pathogen resistance (Agrios, 2005). Efforts have been made to understand the durability of both systems. Qualitative resistance is the most straightforward and simple method for breeders, but it is also the least durable. Resistance genes can break down in as few as three years if pathogen loads are sufficiently high (Kiyosawa, 1982). Quantitative resistance is much more difficult to utilize, but is much more durable. Since molecular markers are now available for some QTL, efforts to pyramid quantitative traits have become a possibility. As the price of molecular marker technologies drops, as more accurate markers are developed, and as more QTL studies hone in on smaller regions, the opportunity for integrating a more durable breeding scenario into programs increases. Recently, CIMMYT has developed spring wheat breeding materials with quantitative (race non-specific) pyramids of resistance to stem and stripe rust (Singh et al., 2011).

Development of Pyramids with Molecular Markers

The possibility of pyramiding *R* genes has been desired by plant breeders and pathologists alike for a very long time. Early thought around pyramids assumed that the probability of

the pathogen overcoming more than one gene would be impossibly low due to mutation rates (Schafer & Roelfs, 1985). Over time, it has become apparent that overcoming host resistance for one gene is not necessarily independent of the other genes (Mundt, 1990). The desire for these pyramids still exists because, in spite of pathogens' abilities to overcome them, strategic pyramiding can still increase the durability of major *R* genes. The ability to combine multiple *R* genes and different kinds of resistance is a breakthrough in disease resistance breeding. No one can guarantee that a pyramid will be durable permanently, but each QTL and mode of action will extend the "boom" period of a major *R* gene. There are many examples of gene pyramids developed with marker-assisted selection in other crops, like rice, soybean, lentil (Huang et al., 1997; Tar'an et al., 2003; Joseph et al., 2004; Shi et al., 2009), and a few in wheat (Barloy et al., 2007; Mago et al., 2011b). These examples include pyramids of viral, fungal, nematode, and bacterial pathogen resistance genes.

Quantitative resistance traits can be pyramided without molecular markers in the absence of a major resistance gene. Multigenic resistance is more durable and, sometimes, incomplete. Sufficient resistance to stem rust has been demonstrated by pyramiding four to five minor genes (Knott, 1982). Major gene pyramids can be durable for many years, versus single gene resistance, which often breaks down in 3-5 years (Parlevliet, 2002). While resistance of many 4-5 *Sr* gene pyramids has broken down, evidence suggests that the genes selected for the pyramid are more important than the number of genes (Green & Campbell, 1979; Mundt, 1990). It is also important to consider if the genes being used in the pyramid have been deployed as single major genes on a large scale. *R* genes that target a pathogen

site more important for pathogen fitness tend to be more durable because there is a higher fitness cost for the trait's loss. Certain genes enhance the major gene resistance of other diseases, for example *Lr34* for leaf rust resistance enhances stem rust resistance QTL (Hiebert et al., 2011; Kolmer et al., 2011). Using major and minor genes, quantitative traits, and *R* gene enhancers in well-coordinated pyramids should help breeders deploy more durable resistance than ever seen in modern breeding.

Pyramiding Linked Genes

Scientists utilize the process of recombination during meiosis to map the physical location of a gene or quantitative trait locus (QTL) to a phenotype of interest, like disease resistance (Buerstmayr et al., 2009). The closer two loci are to each other on a chromosome, the probability of them staying together in the progeny is higher. The physical closeness of genetic loci is called linkage. If you have a truly random mating population, which does not occur in agriculture, the further two loci on a single chromosome are from each other, the lower their levels of linkage disequilibrium are expected to be. If a physical or genetic map of molecular markers is available, we are able to identify the region where a gene lies by identifying markers in high linkage disequilibrium with the phenotype of interest (Lynch & Walsh, 1998).

When a plant breeder crosses two parents to recover a desired genetic trait from each parent in the same progeny, but both traits are in nearly the same genetic location on the chromosome, recovery of both traits in one gamete is very low. The recovery is low because

the two traits, just like a marker and a QTL, are linked. Recovery of them together is nearly impossible because the probability of recombination during meiosis between the two closely loci is very low. Standard size breeding populations are often not large enough to detect a break in recombination. This situation where two traits in the same chromosomal region in different plants cannot be combined in one gamete is called repulsion linkage; when they cannot be broken apart it is called coupling (Lynch & Walsh, 1998).

In this situation, molecular markers can be utilized to identify breaks in the linkage. Molecular markers can be used to "break" repulsion linkage if 1) they are available for both traits and 2) the markers are more tightly linked to their respective trait that the two traits or two markers are linked to each other. The F_2 population of the parental cross needs sufficient size for the probability of recombination between the two loci to be met. Molecular markers could assist us in breaking repulsion linkage between *Fhb1* and *Sr2* on chromosome 3BS since they both have tightly linked markers.

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Tables

Table 1. Stem rust (*Sr*) genes effective and ineffective against race TTKSK, adapted from Singh et al. (2008).

Origin of *Sr* genes	Ineffective	Effective
Triticum aestivum	*5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f, 10, 15, 16, 18, 19, 20, 23, 30, 41, 42, Wld-1*	*28, 29, 48, Tmp, Web, Cad*
Aegilops comosa	*34*	
Aegilops searsii		*51*
Aegilops speltoides		*32, 39, 47*
Aegilops tauschii		*33, 45, 46*
Aegilops ventricosa	*38*	
Dasypyrum villosum		*52*
Secale cereale	*31*	*27, 1A.1R*
Thinopyrum elongatum		*24, 25, 26, 43*
Thinopyrum intermedium		*44*
Triticum araraticum		*40*
Triticum monococcum	*21*	*22, 35*
Triticum timopheevii		*36, 37*
Triticum turgidum	*9d, 9e, 9g, 11, 12, 17*	*2, 13, 14, Web*

Table 2. QTL identified for resistance to Fusarium head blight in tetraploid and hexaploid wheat.

Chr.	QTL Reference
1A	Schmolke et al., 2008
1AL	Semagn et al., 2007
1AS	Jiang et al., 2007a,b
1B	Buerstmayr et al., 2002; Zhou et al., 2004; Steiner et al., 2004; Shen et al. 2003a; Schmolke et al., 2005; Liu et al., 2009; Zhang et al., 2010; Miedaner et al., 2011
1BL	Mardi et al., 2006; (1BL.1RS) Ittu et al., 2000; Semagn et al., 2007; Häberle et al. 2009a
1BS	Klahr et al., 2007
1D	Miedaner et al., 2011
1DL	Yang et al., 2005a
1DS	Ittu et al., 2000; Klahr et al., 2007
2A	Ma et al., 2006a; Steiner et al., 2004; Gervais et al., 2003
2AL	Paillard et al., 2004
2AS	Semagn et al., 2007
2B	Steiner et al., 2004; Gilsinger et al., 2005; Liu et al., 2007; Bonin & Kolb, 2009; Miedaner et al., 2011
2BL	Schmolke et al., 2005; Schmolke et al., 2008
2BS	Gervais et al., 2003; Gilsinger et al., 2005; Somers et al. 2006
2D	Ma et al., 2006b; Jia et al., 2005; Lin et al., 2006; Liu et al., 2009; Zhang et al., 2010; Miedaner et al., 2011
2DL	Somers et al., 2003; Jiang et al., 2007a,b; Mardi et al., 2005
2DS	Shen et al., 2003a; Yang et al., 2005a
3A	Yang et al., 2005a; Steiner et al., 2004; Shen et al. 2003b; Liu et al., 2009; Miedaner et al., 2011
3AL	Mardi et al., 2006; Paillard et al., 2004
3AS	Bourdoncle & Ohm, 2003; Yu et al., 2008; Otto et al., 2002; Chen et al., 2007
3B	Klahr et al., 2007; Liu et al., 2007; Bonin & Kolb, 2009; Zhang et al., 2010
3BC	Yang et al., 2005a
3BL	Bourdoncle & Ohm, 2003; Paillard et al., 2004
3BS	Buerstmayr et al., 2002, 2003a,b; Shen et al., 2003a, Bourdoncle & Ohm, 2003; Somers et al., 2003;Yang et al., 2005a; Chen et al., 2006; Ma et al., 2006a; Jiang et al., 2007a,b; Liu & Anderson, 2003; Guo et al. 2003; Liu et al., 2006; Lemmens et al., 2005; Cuthbert et al., 2006; Lin et al., 2004; Zhou et al., 2004; Jia et al., 2005; Mardi et al., 2005; Ma et al., 2006b; Yu et al., 2008; Yang et al., 2005b; Liu et al., 2009

Table 2 Continued

Chr.	QTL Reference
3D	Shen et al. 2003b
3DL	Yu et al., 2008; Klahr et al., 2007
3DS	Paillard et al., 2004
4AL	Paillard et al., 2004
4B	Jia et al., 2005; Lin et al., 2006; Bonin & Kolb, 2009
4BL	Yang et al., 2005b; Liu et al., 2007
4BS	Somers et al., 2003
4D	Ma et al., 2006b; Miedaner et al., 2011
4DL	Yang et al., 2005a
4DS	Draeger et al., 2007; Srinivasachary et al., 2008
5A	Buerstmayr et al., 2002, 2003a,b; Chen et al., 2006; Lin et al., 2006; Ma et al., 2006a; Steiner et al., 2004; Shen et al. 2003b; Liu et al., 2007; Liu et al., 2009
5AL	Gervais et al., 2003; Paillard et al., 2004
5AS	Somers et al., 2003; Yang et al., 2005a; Jiang et al., 2007a,b;
5B	Jia et al., 2005; Liu et al., 2009; Tamburic-Ilincic et al., 2009
5BL	Bourdoncle & Ohm, 2003; Paillard et al., 2004; Klahr et al., 2007
5DL	Yu et al., 2008; Yang et al., 2005b
6AL	Schmolke et al., 2005; Häberle et al. 2007
6B	Steiner et al., 2004; Liu et al., 2009; Bonin & Kolb, 2009; Zhang et al., 2010
6BS	Shen et al., 2003a; Yang et al., 2005a; Cuthbert et al., 2007; Semagn et al., 2007; Somers et al. 2006
6BL	Draeger et al., 2007
6DL	Paillard et al., 2004
7A	Zhou et al., 2004; Jia et al., 2005; Liu et al., 2009; Zhang et al., 2010; Miedaner et al., 2011
7AC	Jayatilake et al., 2011
7AL	Semagn et al., 2007; Klahr et al., 2007; Kumar et al., 2007
7AS	Mardi et al., 2006
7B	Gilsinger et al., 2005
7BL	Yang et al., 2005a
7BS	Jiang et al., 2007a,b; Schmolke et al., 2005; Häberle et al. 2007; Klahr et al., 2007

CHAPTER II:

Using Molecular Markers to Pyramid Resistance Genes in Eastern Winter Wheat

Abstract

Since the 1930s, the gene *Sr2* has served as a source of durable adult plant resistance to stem rust of wheat. The tetraploid wheat-introgression carrying *Sr2* is located on the short arm of chromosome 3B in the same region as the *Fhb1* locus conferring resistance to Fusarium head blight (FHB). Isolation of *Sr2* and *Fhb1* in the same genotype requires selection for recombination between the resistance genes. This favorable recombination event has been difficult to select for in traditional breeding programs. Selection for resistance to FHB and for adult-plant resistance (APR) to stem rust occurs at later stages of inbreeding due to the quantitative nature of resistance. Appropriately-sized populations would need to be maintained in order to recover lines having *Fhb1* and *Sr2* in coupling. In this research, SSR and SNP markers were used to detect recombination on chromosome 3BS in a population of 319 F_2 plants generated from crosses between eastern winter wheat plants carrying the introgressed genes. The observed linkage distance between the marker *Xumn10* (closely linked to *Fhb1)* and *XcsSr2* (closely linked to *Sr2),* was approximately 10 cM. Fourteen recombinant plants having marker alleles associated with resistance in coupling were identified. Crosses have been made to pyramid the coupled *Fhb1-Sr2* genes with other rust resistance genes in a variety of eastern winter wheat backgrounds. The resulting disease resistant wheat germplasm will be available to breeding programs.

Introduction

The fungal pathogen stem rust, *Puccinia graminis* f. sp. *tritici* Pers. (*Pgt*) (Roelfs and Martens, 1988), has afflicted wheat (*Triticum aestivum* L.) production since the nearly the beginning of wheat cultivation (McIntosh et al., 1995; Peterson, 2001). The last major stem rust epidemic in the U.S.A. was in 1935, with 50% yield losses in Minnesota and North Dakota (Peterson, 2001). Campaigns for the eradication of common barberry (*Berberis vulgaris* L.), the alternate host of stem rust, combined with deployment of host plant resistance and the cultivation of early maturity varieties (Marshall, 1989) have prevented stem rust epidemics in the U.S. for more than 70 years (Peterson, 2001). From 1955-2001, globally there were no stem rust epidemics with more than 5% yield losses reported (Peterson, 2001). Stem rust epidemics were prevented by the global deployment of the *Sr31* resistance gene in CIMMYT germplasm (Singh et al., 2008). However, stem rust re-emerged as a global threat to wheat production in 1998 when a race, Ug99 (TTKS) was detected with virulence to *Sr31* in Uganda (Roelfs & Martens, 1988; Pretorius et al., 2000). Since 2001, variants in the lineage of Ug99 (currently designated as race TTKSK) have been detected with virulence to *Sr24* (race TTKST) and *Sr36* (race TTTSK) (Jin et al., 2008; Jin et al., 2009). The Ug99 lineage of stem rust races is able to overcome all widely-used sources of stem rust resistance in most breeding programs throughout the world (Njau et al., 2010).

The first source of host plant resistance to stem rust in U.S.A. germplasm was introgressed into *T. aestivum* from ‘Yaroslav’ emmer wheat [*T. turgidum* ssp. *dicoccon*,

genome BBAA] into the cultivar ‘Hope’ and the breeding line H44-24 (McFadden, 1930). The source of resistance was identified as a single gene and named *Sr2* (Ausemus et al., 1946; Knott, 1968). *Sr2* was mapped by monosomic analysis to chromosome 3B (Hare and McIntosh, 1979; Spielmeyer et al., 2003). Though ‘Hope’ and H44-24 were not widely cultivated due to the negative agronomic traits associated with the emmer introgression, they have been widely used in breeding programs (McIntosh et al., 1995). Resistance conferred by *Sr2* is recessive and is only effective in adult plants (Ausemus et al., 1946; Knott, 1968). Both qualities make it difficult to phenotype, but *Sr2* is so tightly linked to the trait pseudo-black chaff (PBC) that PBC has been used to effectively detect the presence of *Sr2* (Kota et al., 2006). *Sr2* is the only cataloged source of non-race specific and partial resistance to stem rust (Singh et al., 2006) making it an important source of durable resistance when pyramided with other genes. The single nucleotide polymorphism (SNP) marker *XcsSr2* co-segregated with *Sr2* in a fine mapping population and was predictive for the presence of *Sr2* in diverse germplasm (Mago et al., 2011). Based on screening with *XcSr2*, the resistance gene is not present in contemporary soft winter wheat cultivars and breeding lines from the eastern growing region of the U.S. (Brown-Guedira, unpublished data).

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch] (Goswami and Kistler, 2004), is another fungal disease of wheat of economic importance around the world. Like stem rust, FHB causes losses in grain yield, but may also result in the production of the toxin

deoxynivalenol (DON) that inhibits protein biosynthesis in eukaryotes. DON, when ingested in infected grain by humans or livestock, causes stomach ailments, convulsions, neurological problems, and even death in high doses (Parry et al., 1995). The tolerance levels for DON are only 1 ppm in grain destined for human consumption in the U.S. (FDA, 2010).

One of only three named FHB resistance genes, the *Fhb1* locus on chromosome 3B, is found in the common wheat cultivar 'Sumai3' and related cultivars from China (Wang and Miller, 1988; Waldron et al., 1999; Anderson et al., 2001). Resistance to FHB is quantitative, so no major genes conferring complete FHB resistance are known. The *Fhb1* gene corresponds to the locus on the distal portion of the short arm of chromosome 3B that has been detected in many QTL mapping studies of FHB resistance and was subsequently fine mapped (Cuthbert et al., 2006). Resistance conferred by *Fhb1* has been validated in many studies, but its effect ranges widely, from 5% to 48% reduced FHB spread on the spikelet under controlled conditions (Buerstmayr et al., 2009). The markers *Xumn10* and *Xsnp3BS-8* are approximately 0.6 cM apart, and both are diagnostic for *Fhb1 (*Bernardo et al., 2012). *Xumn10* is most closely linked to *Fhb1* (Bernardo et al., 2012). Unlike *Xsnp3BS-8*, *Xumn10* is not a dominant marker and heterozygous genotypes can be identified (Liu et al., 2008; Bernardo et al., 2012).

Host plant resistance is the most cost-effective way to control FHB and stem rust (Ruckenbauer et al., 2001; McIntosh et al., 1995). Due to their proximities on chromosome 3BS and presence in different germplasm, *Sr2* and *Fhb1* are not found together in cultivars.

Together, the genes could be a valuable component of durable resistance if deployed in cultivars. Our objectives were to isolate *Sr2* and *Fhb1* in coupling in an eastern winter wheat background and to begin introgressing the linked genes into elite eastern winter wheat cultivars.

Materials and Methods

Plant material

Eastern winter wheat germplasm with the resistance genes *Fhb1* and *Sr2*, respectively, were developed at North Carolina State University. Three $BC_2F_{2:3}$ plants, NC06-27-11-13, NC06-27-11-16, and NC06-27-11-19, were the source of *Sr2* in our crosses (Figure 1). These plants were derived from backcrossing the hard red spring wheat cultivar Pavon 76 (PI 519847) to the soft red winter wheat (SRWW) cultivar USG3209 (GSTR 11001) by Dr. J. Paul Murphy (Table 1).

Four F_1 plants, UX0359-10, UX0355-11, UX0356-7, and UX0356-19, were used as the source of *Fhb1* in our crosses. These plants had been developed through a series of crosses to introgress *Fhb1* into winter wheat and to pyramid *Fhb1* with the adult plant rust resistance gene *Lr34/Yr18* (Table 1). The VA01W-476 parent in the pedigree of these lines is a doubled haploid selection made from a cross between the Chinese line W14 and the SRWW cultivar Roane (PI 612958) by Dr. Carl Griffey. The W14 donor of *Fhb1* in this experiment also donated an additional FHB resistance locus, *Qfhs.nau-2D.* The rust resistance locus *Lr34/Yr18* was derived from the line MOI*1019. Additional rust resistance

genes were present in the SRWW parents, including *Sr38/Yr17/Lr37* in SS8641, and stem rust resistance gene *Sr36* in NC-Neuse (PI 633037) and USG3209 (Table 1). The stem, stripe, and leaf rust resistance genes, *Sr38/Yr17/Lr37*, are all linked on a single introgression from *Aegilops ventricosa* Tausch onto *T. aestivum* chromosome 2AS. From this point forward, *Sr38* will be used to refer to the 2AS *Ae. ventricosa* introgression. Marker-assisted selection for these FHB and rust resistance genes was done on F_1 plants at each stage in crossing.

The F_1 progeny from three crosses between plants having *Fhb1* and *Sr2* were genotyped with markers because the F_1 individuals were expected to segregate at the resistance loci. Selected plants heterozygous for both *Fhb1* and *Sr2* were grown and allowed to self-pollinate to produce the F_2 populations used for selection of recombinant gametes (Figure 1). Seed from each F_1 individual was kept separate since plants differed at other loci segregating in the populations. The populations consisted of 90 F_2 progeny from the F_1 plants UX0792-6 and -7 (Pedigree: UX0359-10/NC06-27-11-16), 136 F_2 progeny from the F_1 plants UX0771-2 and -6 (NC06-27-11-19/UX0355-11&UX0356-7), and 93 F_2 progeny from the F_1 plant UX0773-15 (UX0356-19/NC06-27-11-13) (Table 1). Herein, the 319 total F_2 plants from these three populations are referred to as the UX07 population.

All winter wheat plants were grown by germinating seeds on cotton balls in 128-well seedling trays at room temperature. After leaf tissue was harvested from seedlings for DNA isolation, the cotton balls were covered with potting soil and placed in vernalization at 4°C for 8 weeks in a 12 hr light/12 hr dark cycle. The selected plants were transplanted into

one-gallon pots and grown in a greenhouse with short-day conditions (10 hr light/14 hr dark) and in temperatures between 18 and 24 °C. After plants were established, supplemental lighting was used to provide a long-day cycle (16 hr light/8 hr dark). Spring wheat plants are grown in identical conditions except without an 8-week period in vernalization.

Selection of recombinant plants

While in vernalization, the 319 F_2 plants from the UX07 population were genotyped with the markers: *Xumn10*, *Xgwm533*, and *XcsSr2*. F_2 plants with two genotypes were selected: (i) homozygous resistant at *Xumn10* with the presence of the resistance allele at *XcSr2,* which was a dominant marker in our population and (ii) heterozygous at *Xumn10* with the presence of the resistance allele at *XcSr2* and a homozygous resistance genotype at *Xgwm533* (Figure 1). Fourteen plants identified with these genotypes were used for further crossing. Herein, these 14 selected F_2 plants are referred to as the 14 recombinants. The 14 selected recombinants were also genotyped with markers for *Xgwm493, Xsnp3BS-7, Xgwm389, Sr38, Lr46/Yr29, Lr34/Yr18, Sr36,* and *Qfhs.nau-2DL*. The 14 recombinants have variable combinations of the resistance genes *Sr38, Lr46/Yr29, Lr34/Yr18, Sr36,* and *Qfhs.nau-2DL* in homozygous and heterozygous genotypes. At harvest the individual plants' $F_{2:3}$ seed was kept separate due to their unique recombination events and variable combinations of FHB and rust resistance genes.

Development of populations for phenotypic verification

The 14 recombinants were transplanted into one-gallon pots in the greenhouse after 8 weeks of vernalization at 4°C, along with vernalized plants of the cultivars Roane and SS8641 and two week old plants of the spring line LMPG-6. Roane is a *Pgt*-susceptible winter wheat with moderate resistance to FHB. LMPG-6 is a spring wheat line with susceptibility to both *Pgt* and FHB. SS8641 is an FHB susceptible winter cultivar with *Pgt*-resistance from *Sr38*. These genotypes were used as female parents in crossing with the 14 recombinants to obtain the recombinant *Fhb1-Sr2* gametes in a stem rust and/or FHB susceptible genetic background (Figure 2). These testcrosses were developed to confirm the presence of *Fhb1* and *Sr2* with FHB and *Pgt*-screening. Verification of *Pgt* resistance could not be done in selfed generations of the recombinants because all 14 were homozygous for the resistance gene *Sr36*. All F_1 progeny of the Roane, LMPG-6, and SS8641 testcrosses were genotyped. Selected plants with heterozygous *Fhb1* and *Sr2* genotypes (*Fhb1fhb1Sr2sr2*) were self-pollinated (Figure 2). The F_2 progeny were genotyped and homozygous resistant (*Fhb1Fhb1Sr2Sr2*) and homozygous susceptible (*fhb1fhb1sr2sr2*) plants were selected and self-pollinated (Figure 2). $F_{2:3}$ seed from each individual was maintained separately.

Development of population for selection of disease resistant germplasm

At the same time Roane, SS8641, and the UX07 population were planted, 11 eastern U.S. soft winter wheat (SWW) genotypes were planted and placed into vernalization for 8 wks. The 11 cultivars and breeding lines were: AGS 2020, AGS 2035, AGS 2060, Caledonia R,

IL00-8530, IL00-8633, INW07031, McCormick, NC-Neuse, Shirley, and VA01W205. These genotypes have varying stem rust and FHB resistance levels but do not carry *Sr2* nor *Fhb1*. These 11 SWW genotypes were used as female parents in crosses with the 14 recombinants (Figure 3) in January 2011. The resulting F_1 progeny were genotyped. Selections for *Fhb1fhb1Sr2sr2* genotypes were made, plants self-pollinated, and seed harvested (Figure 3).

Genotyping

Genomic DNA was isolated from the green tissue of single F_1 and F_2 seedlings, five-plant bulk samples of the UX07 population parents and of cultivars Pavon 76, VA01W-476, USG 3209, SS8641, and NC-Neuse. Tissue was harvested to 96-well plates and stored at -80°C. The frozen tissue was macerated with steel beads with a GenoGrinder 2000 (SPEX CertiPrep, Metuchen, NJ). DNA extractions were performed according to a modified SDS protocol originally published in Pallotta et al. (2003).

Single-nucleotide polymorphism (SNP), sequence-tagged site (STS), and simple sequence repeat (SSR) markers were used for detection of *Fhb1* (*Xumn10*, *Xsnp3BS-8*, *Xsnp3BS-7*) and *Sr2* (*Xgwm533*, *XcsSr2*) (Table 2). Plants were also genotyped with linked SSR markers to determine the presence of *Qfhs.nau-2DL* (*Xcfd233*, *Xgwm608*), *Sr36* (*Xwmc477*), and with SNP markers linked to *Yr17*/*Lr37*/*Sr38*, *Lr46*/*Yr29*, *Lr34*/*Yr18* (Table 3).

Polymerase chain reaction (PCR) amplification of SSR and STS markers was performed in a 12 µL reaction volume consisting of 2 µL (20 ngμL^{-1}) genomic DNA template, 1.20 µl 10X PCR buffer (10 mM Tris-HCL, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 0.96 µl 2.5 mM mixture of dNTPs, 0.30 µl forward primer (5 µM), 0.30 µl reverse primer, 0.09 µl of *Taq* polymerase, and 7.15 µl of water, bringing the reaction total to 12 µl. The forward primers were each labeled with one fluorescent dye (6-FAM, VIC, HEX, or NED). Thermal cycler PCR conditions were: (i) initial denaturation at 95°C for 2 min; (ii) 35 amplification cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C; (iii) 5 min final extension at 72°C; (iv) 4°C hold. Amplifications were performed using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Separation of fragments was performed by capillary electrophoresis using an ABI3130xl Genetic Analyzer (Applied BioSystems, Foster City, CA). Data were analyzed using GeneMarker 1.85 software (SoftGenetics, State College, PA).

SNP markers were assayed using KASPar assays (Table 1). Reactions were run according to manufacturer's instructions (KBioscience Ltd., Hoddeson, UK). A Roche Lightcycler® 480 Real-Time PCR instrument and software were used for endpoint genotyping (F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Genetic distance and linkage analysis was conducted using JoinMap 3.0 software (Kyazma B.V., Wageningen, Netherlands), based on a Kosambi's mapping function with an LOD value of 9.0.

Stem rust screening

Urediniospores of North American race QFCS were collected directly off plants of the susceptible cultivar McNair 701 (CI 15288) and suspended in Soltrol 170 light mineral oil. Fourteen days after planting, seedlings from F_2 testcrosses to Roane and LMPG were misted with the suspension using an airbrush in well-ventilated conditions. After waiting 20 minutes for the oil to evaporate, the trays were placed in dew chambers in 22 hrs darkness, with 34°C water and 6°C wall temperatures at 80% humidity. The chamber was shut off for two hours. After 24 hours in the dew chamber, trays were removed to greenhouse, and scored for reactions to stem rust 21 days later.

FHB screening

In sterile conditions, three isolates of *F. graminearum* from North Carolina were cultured on synthetic nutrient agarose plates at room temperature. These three isolates were mixed in a suspension of sterile water to a concentration of 50,000 spores/mL, kept at 4°C. At anthesis, plants were inoculated with an injection of 10 µl of the suspension into one floret halfway up the spikelet. Inoculated plants were immediately placed in a misting chamber for 72 hours. The misting chamber was exposed to ambient light, under a misting cycle of 8-hours day with one 30-second mist every 150 sec. and 16-hours night with one 30-second mist every 300 sec. At the end of 72 hr, inoculated plants were placed in the greenhouse and 21 days after inoculation FHB spread was recorded as a fraction of infected spikes per total

spikes per inoculated head. Data was analyzed with SAS 9.2 software (SAS Institute Inc., Cary, North Carolina, USA), running a general linear model.

Results

Marker evaluation of parents and populations

Plants NC06-27-11-13, NC06-27-11-16, and NC06-27-11-19 were homozygous for the resistant allele (G) when genotyped with marker *XcsSr2* that co-segregates with *Sr2* (Table 4). Plants UX0359-10, UX0355-11, UX0356-7, and UX0356-19 amplified the susceptible allele (C) with marker *XcsSr2*, indicating they are heterozygous for C and null alleles present in their parents NC-Neuse and VA01W-476's genotypes. When genotyped with marker *Xumn10*, which co-segregates with *Fhb1* (Liu et al 2008), UX0359-10, UX0355-11, UX0356-7, and UX0356-19 were determined to be heterozygous (Table 4). Plants NC06-27-11-13, NC06-27-11-16 and NC06-27-11-19 were homozygous for the 236 bp fragment associated with the absence of the *Fhb1* resistance allele (Table 2, Table 4).

Parents were evaluated with additional markers previously mapped to the *Fhb1* and *Sr2* region, including *Xgwm493, Xsnp3BS-7* and *Xsnp3BS-8* located proximal to *Fhb1, Xgwm533* that is located between *Xumn10* and *XcsSr2* and marker *Xgwm389* that is distal to *Sr2* (Paux et al., 2008; Bernardo et al, 2012; Figure 4b). These flanking markers were polymorphic among the lines tested (Table 4).

UX0359-10, UX0355-11, UX0356-7, and UX0356-19 were determined to be heterozygotes with marker *Xgwm533*. These plants were also heterozygous for markers

Xsnp3BS-8and *Xsnp3BS-7* proximal to *Fhb1* (Table 4). The plants were homozygous for the 195 base pair allele at *Xgwm493* that is common between VA01W-476 and the three SRWW cultivars in the pedigree of this germplasm. When evaluated with the *XcsSr2* marker, the C allele was detected in UX0359-10, UX0355-11, UX0356-7, and UX0356-19. Distal to *Sr2*, UX0359-10, UX0355-11, UX0356-7 and -19 were heterozygous for alleles at marker *Xgwm389* derived from NC-Neuse (115 bp) or USG3209 and SS8641 (139 bp). These genotypes indicate that in the parental gametes having *Fhb1*, the region distal to *XcsSr2* was derived from the SRWW parent NC-Neuse while the region between *XcsSr2* and *Xsnp3BS-8* was derived from the W14 donor of *Fhb1* (Table 4). We were unable to determine the origin on the region proximal to *Xsnp3BS-8* due to lack of polymorphism between VA01W-476, W14, NC-Neuse, SS8641, and USG3209.

Plants NC06-27-11-13, NC06-27-11-16 and NC06-27-11-19 were homozygous for the 236 bp allele of *Xumn10* and the 118 bp allele of *Xgwm533* that were not polymorphic between the Pavon 76 and USG3209 parents. The plants were homozygous for the G allele of *XcsSr2* derived from the *Sr2* donor parent Pavon 76 (Table 4). Plants NC06-27-11-13, -16 and -19 carry the 465 bp allele of marker *Xsnp3BS-7* and the C allele of marker *Xsnp3BS-8* that are present in the USG3209 parent. Pavon 76 has null alleles at *Xsnp3BS-7* and *Xsnp3BS-8*. The proximal marker *Xgwm493* was polymorphic between Pavon 76 and USG3209 and heterozygous in the NC06 parents. Thus, we imputed that the NC06 plants used for crossing were heterozygous at *Xsnp3BS-7* and *Xsnp3BS-8* (Table 4).

Parent plants were also genotyped for markers associated with other rust and FHB resistance loci known to be segregating in these materials. NC06-27-11-13, -16, and -19 were homozygous for the *Lr46/Yr29* gene that confers adult plant resistance to leaf rust and stripe rust and the *Sr36* gene conferring seedling resistance to stem rust (Table 5). Plants UX0359-10, UX0355-11, UX0356-7, and UX0356-19 were heterozygous for *Lr34/Yr18*. UX0355-11, UX0356-7, and UX0356-19 were heterozygous for *Qfhs.nau-2DL* and homozygous for *Sr36.* UX0356-7 and UX0356-19 were also heterozygous for *Sr38.* Plant UX0359-10 was homozygous for *Sr38* and heterozygous for the *Sr36* gene, but did not carry the *Qfhs.nau-2DL* resistance QTL (Table 5).

Recombination detection

Recombination was observed between markers in the *Fhb1* and *Sr2* region. Fifty-three recombination events between *Xumn10* and *XcsSr2* were detected in the population of 319 F_2 plants. Marker order of the linkage map generated with the UX07 population (Figure 4a) is in agreement with the order of previous reports (Bernardo et al., 2012, Figure 4b). The observed distance in our map between markers *XcsSr2* and *Xgwm533* was 3.3 cM and between *Xumn10* and *Xgwm533* is 7.2 cM, placing *Xgwm533* much closer to *Sr2* than to *Fhb1*. The observed linkage distance between *Xumn10* and *XcsSr2* was 10.5 cM. Marker *Xsnp3BS-8* was 1.3 cM proximal to *Xumn10* (Figure 4a).

Sixteen individuals were identified having marker alleles that indicated resistance genes *Fhb1* and *Sr2* in coupling. Fourteen of the individuals were used for further crossing.

Ten recombinants have an imputed homozygous *Fhb1* and heterozygous *Sr2* genotype (Table 5). Of those 10, seven have a recombination breakpoint between *Xumn10* and *Xgwm533* (Table 4). In the other three recombinant plants, recombination occurred between *Xgwm533* and *XcsSr2* (Table 4). Four recombinants have an imputed heterozygous *Fhb1* and homozygous *Sr2* genotype, all with a recombination breakpoint between *Xumn10* and *Xgwm533* (Table 4). Since the *XcsSr2* marker is dominant in our population, we imputed heterozygous genotypes for *Sr2* based on *Xgwm533* genotypes. *Fhb1* genotypes were based on the co-dominant marker *Xumn10*. Markers *snp3BS-7* and *snp3BS-8* were used to determine if additional recombination had occurred proximal to *Xumn10*. However, these markers were of limited usefulness since both have null alleles in the Pavon 76 and NC06 parents. Evaluation of recombinants with *Xgwm493* indicates that the region proximal to *Xumn10* was likely derived from the VA01W-476 parent in all the recombinant gametes. Varying combinations of markers linked to other rust and FHB resistance genes were found in the recombinants. All had at least one copy of *Sr36*, and at least one or more of the following resistance genes: *Sr38/Yr17/Lr37*, *Lr46/Yr29*, *Lr34/Yr18*, and *Qfhs.nau-2DL* (Table 4). Two of the recombinants had all five resistance genes, eight had four resistance genes, and the remaining three had two or three resistance genes in the homozygous or heterozygous state (Table 4).

Stem rust screening

Efforts to validate the presence of *Sr2* and *Fhb1* in the selfed progeny of recombinant plants by disease screening is complicated by the presence of other resistance genes, including the *Sr36* gene conferring seedling resistance to stem rust that was homozygous in 13 of the 14 *Fhb1-Sr2* recombinants (Table 5). In an attempt to develop populations for verification of APR to stem rust, the 14 recombinant plants were crossed to LMPG-6 and/or Roane, neither of which carries a known stem rust resistance gene (Figure 2). F_2 populations consisting of 632 and 559 seedlings from the LMPG-6 and Roane crosses respectively, were genotyped with markers *Xumn10* and *XcsSr2*. Seedlings were also screened with stem rust race QFCS in order to identify susceptible plants that do not carry the *Sr36* resistance gene. Of the 1,191 total plants genotyped, 214 were homozygous at the *Xumn10* and *XcsSr2* loci (χ^2=9.1, p=0.01). Of these, only 27 were determined to be seedling susceptible to stem rust (χ^2=17.5, p<0.001). This excess of seedling stem rust resistant plants observed in our populations is consistent with reported preferential transmission observed for *Sr36* (Allard & Shands, 1954).

Individuals that were seedling susceptible to stem rust and homozygous resistant (*Fhb1Fhb1Sr2Sr2*) or homozygous susceptible (*fhb1fhb1sr2sr2*) for the recombinant gametes were selected. Twenty-two and 13 F_2 plants from the crosses with LMPG-6 and Roane, respectively, homozygous for both resistance genes were selected. Seventeen and 13 F_2 plants from crosses with LMPG-6 and Roane, respectively, homozygous for both susceptible alleles were also selected. All 65 selections had susceptible genotypes when

evaluated with the *Xwmc477* SSR markers linked to *Sr36.* Due to severe powdery mildew infection of seedlings, plants were treated with fungicide and could not be tested for adult-plant reaction to stem rust. The F_2 selections were grown to maturity in the greenhouse and pseudo-black chaff (PBC) was seen on many of the plants thought to carry *Sr2* (Figure 5). Large amounts of $F_{2:3}$ seed harvested from these 65 lines are available to compare reaction types of plants with contrasting marker genotypes to confirm the presence of *Sr2* by screening in the field for adult stem rust resistance (Figure 2).

FHB screening

Three F_1 plants from crosses between recombinant plants and the FHB susceptible cultivar SS8641 that were determined to have *Fhb1fhb1Sr2sr2* genotype and lacking resistance markers for *Qfhs.nau-2DL* were used to produce an F_2 population of 123 plants. Genotyping of the F_2 seedlings identified 11 and 10 having *Fhb1Fhb1Sr2Sr2* and *fhb1fhb1sr2sr2* genotypes, respectively. These seedlings were transplanted into the greenhouse, and we were able to inoculate between one and four heads on each of these 21 plants at flowering. Overall, significant differences in severity were not observed between *Fhb1Fhb1Sr2Sr2* and *fhb1fhb1sr2sr2* genotypes in combined analysis of all inoculated plants (p=0.23). The overall mean percentage of infected spikelets for plants homozygous for the *Fhb1* allele at the *Xumn10* locus (n=11) was 25% compared to 36% for plants homozygous for the allele associated with susceptibility (n=10). When data from the 13 plants derived from crosses between recombinant UX0792-6-17 and SS8641 were analyzed separately, differences were

observed between plants with and without *Fhb1*. The mean percentage infected spikelets was 30% for *Fhb1Fhb1* genotype plants and 50% for *fhb1fhb1* genotype plants (p=0.04). There was great variation in the amount of susceptibility even per plant (Figure 6).

Sr2-Fhb1 germplasm development

Fifty-five crosses were made with 11 eastern U.S. SWW cultivars and breeding lines. A total of 883 F_1 plants from these crosses were genotyped with markers for *Fhb1*, *Sr2* and other resistance genes segregating in these germplasm. One-hundred and seventy plants were selected with the heterozygous genotype (*Fhb1fhb1Sr2sr2*) and heterozygous for three or more of the other resistance genes. There are currently over 11,600 F_2 seeds harvested from the 170 selections from which homozygous resistant lines can be selected.

Discussion

Recovering plants having two partial resistance genes when those genes are in repulsion linkage can be a difficult task when the only tools available are phenotypic evaluation. However, the use of closely linked markers to select favorable recombination events can be done on large numbers of individuals at any stage of inbreeding. Our ability to focus sufficient genotyping resources on analyzing a large population in the seedling stage allowed us to reduce the resources in time and space for identifying and growing out individual plants carrying recombinant gametes. An additional advantage of marker-assisted selection is that the same technology can be used to identify genes affecting multiple traits,

including resistance to different diseases. While identifying and selecting for quantitative resistance to multiple diseases requires large quantities of seed for replicated testing of inbred material in a traditional program, the use of diagnostic DNA markers allowed us to determine the presence of minor effect genes and QTL like *Fhb1, Sr2, Lr46/Yr29* and *Lr34/Yr18* in combination with major resistance genes. With the use of predictive DNA markers, the effect of the partial rust resistance genes will be missed in the field-based evaluations since major resistance genes *Sr36* and *Yr17/Lr37/Sr38* were also present in many plants.

The genotyping results of this project made it clear that the presence of null alleles causes difficulties in detecting recombinants. It is important to have familiarity with the genotypes of the parents in the pedigrees of the population's parents before making assumptions about the presence of recombinants. In the F_2 generation, the signature of a recombinant is heterozygosity at one resistance locus and homozygosity at the other locus. It is very simple to accidentally select heterozygotes that appear to have this genetic signature because there are unknown null alleles present at one of the loci. Although marker *XcsSr2* co-segregates with the *Sr2* resistance gene, we relied on the linked SSR marker *Xgwm533* to impute the *XcsSr2* genotype.

Our goal was to develop germplasm adapted to the eastern soft wheat growing regions that have the *Fhb1* and *Sr2* resistance genes so that breeders can deploy these genes as a linkage block. To date, *Fhb1* has limited use in eastern wheat cultivars. Part of the reason *Fhb1* has not been widely deployed, other than the fundamental trouble to cross with

Asian materials, is the perception among wheat breeders that there is linkage drag associated with the unadapted introgression from Chinese wheat. Recently, researchers developed *Fhb1* near-isogenic lines of the wheat cultivar 'Clark.' They reported no yield loss associated linkage drag from the Chinese background of *Fhb1* (Bernardo et al., 2011). Minimizing the percentage of Chinese background was our goal when retrieving *Fhb1* from crosses between VA01W-476 and SWW cultivars when selecting the parents for creating the population for isolation of a *Sr2-Fhb1* recombinant. However, genotyping of the *Fhb1* donor parents of the cross (UX0359-10, UX0355-11, UX0356-7, and UX0356-19) indicates that the region from marker *Xgwm493* to *XcsSr2* was derived from the Asian line W14. Until recently, MAS for *Fhb1* involved the use of flanking markers, which has resulted in the introgression of relatively large segments of chromatin from unadapted germplasm. Selection for *Fhb1*, with incomplete effects that vary in efficacy by cultivar background and environmental conditions, is typically done with linked markers prior to disease evaluation, virtually eliminating the possibility of recovering a smaller chromosome segment from the unadapted parent. With the development of the *Xumn10* marker by Liu et al. (2008), one can select for recombination events in the *Fhb1* region of 3BS.

Although resistance gene *Sr2* was introduced into SWW cultivars in the 1930s, genotyping of hundreds of SWW lines with *XcsSr2* indicate that this gene is not present in contemporary Eastern wheat germplasm (Brown-Guedira, unpublished results). We speculate that *Sr2* has been removed from breeders' programs in the eastern United States due to control of stem rust by major genes for many decades. In addition, PBC expression

can also easily be mistaken as *Stagonospora* glume blotch symptoms, which is a serious disease problem in the warm, humid parts of the Midwestern and Mid-Atlantic states.

When using germplasm developed in this study, there is a possibility that breeders will be able to select for both *Fhb1-Sr2* with proper identification of PBC. While one report failed to dissociate *Sr2* with PBC (Kota et al., 2006), another was able to break their linkage (Mishra et al., 2005). We saw PBC in only some of the recombinant lines, possibly due to environmental influences, or because during recombination between *Sr2* and *Fhb1* also resulted in recombination between *Sr2* and PBC linkage. As breeders have failed to identify a marker for PBC separate from *Sr2*, we can only assume that the variation observed in this study was due to an environmental effect rather than recombination.

It is a long-standing interest of plant breeders and pathologists to develop a simpler way to deploy quantitative resistance. Both *Sr2* and *Fhb1*, with relatively large effects, fall on the border between true quantitative and major effect resistance. The success of isolating recombinants in this project gives us hope that we will more efficiently pyramid QTL for disease resistance as more DNA markers tightly-linked to QTL are developed and validated. *Sr2* offers incomplete but durable resistance, and its presence in eastern U.S. germplasm could increase the durability of the major stem rust resistance genes with which it is pyramided. However, the deployment of *Fhb1* into cultivars currently prevents the simultaneous deployment of *Sr2*. Our hope is that development of germplasm having both genes will increase the use of *Sr2* in regions where FHB is a constraint to production.

Future work

Another season is necessary to validate *Sr2* on the recombinant gametes through screening of adult plants in the field. My proposal is to screen material in Kenya. Great strides have been made for effectively screening winter wheat in Kenya, and the eastern germplasm with other *Sr* genes in their pedigrees will be most useful if screened with the Ug99 family of races.

In the 2012-2013 growing season, the F_2 progeny from crosses between the lines having recombinant gametes and a regionally adapted cultivar will be evaluated in the field for general plant type. The best populations will be harvested and their progeny will be genotyped to identify homozygous $F_{2:3}$ plants having *Fhb1* and *Sr2* in coupling linkage. Good plant types, confirmed homozygous for the recombination event, will be made available to wheat breeding programs. Selections distributed as germplasm should also be evaluated in uniform regional FHB nurseries and in Kenya for *Pgt* in 2013-2014 to confirm disease reactions. Lastly the linkage of the traits will need to be monitored, but the promise remains for a two gene-one marker assay to be made available for eastern winter wheat breeders. It will take many decades to determine if the quantitative traits will increase the longevity of major resistance in resistance pyramids.

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Tables

Table 1. Pedigree and generation information on parents for *Fhb1-Sr2* coupling population. Pedigree and generation information are indicated for the plant used in the cross.

Parents	Generation	Pedigree
NC06-27-11-13	$BC_2F_{2:3}$	USG3209 *(Sr36)**3/Pavon 76 *(Sr2, Lr46/Yr29*)
NC06-27-11-16	$BC_2F_{2:3}$	USG3209 *(Sr36)**3/Pavon 76 *(Sr2, Lr46/Yr29*)
NC06-27-11-19	$BC_2F_{2:3}$	USG3209 *(Sr36)**3/Pavon 76 *(Sr2, Lr46/Yr29*)
UX0359-10	F_1	SS8641(*Sr38)*/4/MOI*1019 *(Lr34/Yr18)*/3/SS8641/NC-Neuse *(Sr36)**2/VA01W-476 *(Fhb1-Fhb2D)*
UX0355-11	F_1	USG3209 *(Sr36)*/4/MOI*1019 *(Lr34/Yr18)*/3/SS8641 *(Sr38)*//NC-Neuse *(Sr36)**2/VA01W-476 *(Fhb1-Fhb2D)*
UX0356-7	F_1	USG3209 *(Sr36)*/4/MOI*1019 *(Lr34/Yr18)*/3/SS8641 *(Sr38)*//NC-Neuse *(Sr36)**2/VA01W-476 *(Fhb1-Fhb2D)*
UX0356-19	F_1	USG3209 *(Sr36)*/4/MOI*1019 *(Lr34/Yr18)*/3/SS8641 *(Sr38)*//NC-Neuse *(Sr36)**2/VA01W-476 *(Fhb1-Fhb2D)*
UX0771-2 & -6	F_1	NC06-27-11-19 (*Sr2, Lr46/Yr29, Sr36*)/UX0355-11&UX0356-7 (*Fhb1-Fhb2D, Sr38, Lr34/Yr18, Sr36*)
UX0773-15	F_1	UX0356-19 (*Fhb1-Fhb2D, Sr38, Lr34/Yr18, Sr36*)/NC06-27-11-13 (*Sr2, Lr46/Yr29, Sr36*)
UX0792-6 & -7	F_1	UX0359-10 (*Fhb1, Sr38, Lr34/Yr18, Sr36*)/NC06-27-11-16 (*Sr2, Lr46/Yr29, Sr36*)

Table 2. Genetic markers used to detect recombination on chromosome 3BS, the markers' locations in relationship to *Fhb1* and *Sr2*, their allele sizes on an ABI 3130xl or KASP polymorphism. Markers gwm493 and gwm389 do not have true resistance and susceptibility alleles, as they are not tightly linked to resistance genes. With gwm389, the resistance allele indicates the allele associated with Pavon 76 and, with gwm493, the resistance allele is the allele associated with all parents except Pavon 76. *indicates *Sr2* resistance allele.

Locus	Marker name	Resistance allele	Susceptible allele(s)	References
Proximal to *Fhb1*	gwm493	195 bp	138 bp	Paux et al., 2008; Bernardo et al., 2012
Proximal to *Fhb1*	snp3BS-8	G	C/null	Bernardo et al., 2012
Proximal to *Fhb1*	snp3BS-7	474 bp	465 bp	Bernardo et al., 2012
Fhb1	snp3BS-11 (Xumn10)	239 bp	236 bp	Liu et al., 2008; Bernardo et al., 2012
Distal to *Fhb1*, proximal to *Sr2*	gwm533	*118 bp	142 bp	Spielmeyer et al., 2003
Sr2	csSr2	A	G/null	Mago et al., 2011
Distal to *Sr2*	gwm389	139 bp	115 bp	Paux et al., 2008; Bernardo et al., 2012

Table 3. Linked markers of other stem rust (*Sr*), leaf rust (*Lr*), stripe rust (*Yr*), and FHB resistance genes and their allele sizes on the ABI 3130xl or KASP polymorphism.

Locus	Marker name	Resistance allele	Susceptible allele(s)	Reference
Sr38/Yr17/Lr37	Lr37-KASP (VENTRIUP-LN2)	G	A	Brown-Guedira, unpublished data (Helguera et al., 2003)
Lr46/Yr29	JF2-2	C	G	Brown-Guedira, unpub.
Lr34/Yr18	Lr34-KASP (XcsLV34)	---	TTC	Brown-Guedira, unpub. (Lagudah et al., 2006)
Sr36	wmc477	186	157	Tsilo et al., 2008
Qfhs.nau-2DL (Fhb-2D) flanking	cfd233	276	272, 280	Jiang et al., 2007; Mardi, 2005
Qfhs.nau-2DL (Fhb-2D) flanking	gwm608	152	156	Jiang et al., 2007; Mardi, 2005

Table 4. Marker data for chromosome 3BS of the UX07 population parents and the 14 F_2 plants of interest. The progenitors of the UX07 parents have unique color codes, identified in the first section. The *Sr2* and *Fhb1* parents' alleles are colored to indicate their parental origin. The recombinant F_2 plants are color-coded with their imputed ancestry. Pavon 76 was used as our source of *Sr2*, and VA01W-476 as *Fhb1*. The progenitors have only one line listed because they have homozygous genotypes, implying that their gametes are identical in this section of the genome. All other parent and recombinant lines are two lines, each representing the unique genotype of each gamete. The parents' genotypes are repeated before their respective progeny. In the recombinants, the recombination breakpoint is indicated with a vertical bar crossing the recombinant gamete.

Linked Marker	gwm493	snp 3BS-8	snp 3BS-7	umn10	gwm 533	csSr2	gwm 389
Pavon76	138	null	null	236	118	G	115
USG3209	195	C	465	236	118	C	139
SS8641	195	C	465	236	118	C	139
VA01W-476	195	G	474	239	142	null	133
NC-Neuse	195	C	null	236	118	null	115
***Sr2* Parents**							
NC06-27-11-13, -16, & -19	138	null	null	236	118	G	115
	195	C	465	236	118	G	139
***Fhb1* Parents**							
UX0355-11, UX0359-10, UX0356-7 & -19	195	G	474	239	142	null	115
	195	C	465	236	118	C	139
Recombinant F_2 Plants							
UX0771-2-10	195	G	474	239 |	118	G	115
	195	G	474	239	142	null	115
UX0771-2-102	195	null	null	236	118	G	115
	195	G	474	239 |	118	G	115
UX0771-2-104	195	null	null	236	118	G	115
	195	G	474	239 |	118	G	115
UX0771-2-99	195	G	474	239	142 |	G	115
	195	G	474	239	142	null	115
UX0771-6-7	195	G	474	239 |	118	G	115
	195	G	474	239	142	null	115
UX0773-15-2	138	null	null	236	118	G	115
	195	G	474	239 |	118	G	115

Table 4 Continued

Linked Marker	gwm493	snp 3BS-8	snp 3BS-7	umn10	gwm 533	csSr2	gwm 389
UX0773-15-56	138	null	null	236	118	G	115
	195	G	474	239	118	G	115
UX0773-15-59	195	G	474	239	118	G	115
	195	G	474	239	142	null	115
UX0773-15-61	195	G	474	239	142	G	115
	195	G	474	239	142	null	115
UX0773-15-62	195	G	474	239	118	G	115
	195	G	474	239	142	null	115
UX0792-6-1	195	G	474	239	142	G	139
	195	G	474	239	142	null	115
UX0792-6-17	195	G	474	239	118	G	139
	195	G	474	239	142	null	115
UX0792-7-53	195	G	474	239	118	G	139
	195	G	474	239	142	null	115
UX0792-7-56	195	G	474	239	118	G	139
	195	G	474	239	142	null	115

Table 5. Presence of wheat rust and scab resistance genes in the UX07 population parents and recombinant progeny. R indicates a homozygous genotype for the resistance allele; S, a homozygous susceptible genotype; and H, a heterozygous genotype. R/H indicates a homozygous resistant genotype in the presence of null alleles; the letter in bold signifies the imputed genotype from flanking marker data.

Gene	***Fhb1***	***Sr2***	***Sr38***	***Lr46/Yr29***	***Lr34/Yr18***	***Sr36***	***Qfhs.nau-2DL***
Chromosome	2BS	2BS	2AS	1BL	7DS	2BS	2DL
Sr2 Parents							
NC06-27-11-13	S	R	S	R	S	R	S
NC06-27-11-16	S	R	S	R	S	R	S
NC06-27-11-19	S	H	S	R	S	R	S
Fhb1 Parents							
UX0359-10	H	S	R	S	H	H	S
UX0355-11	H	S	H	S	H	R	H
UX0356-7	H	S	S	S	H	R	H
UX0356-19	H	S	H	S	H	R	H
Recombinant F_2 Plants							
UX0773-15-59	R	R/**H**	H	H	S	R	R
UX0792-6-17	R	R/**H**	H	R	R	H	S
UX0792-7-53	R	R/**H**	H	R	H	R	S
UX0792-7-56	R	R/**H**	S	R	S	R	S
UX0771-2-10	R	R/**H**	R	S	R	R	H
UX0771-6-7	A	R/**H**	S	R	R	R	R
UX0773-15-62	R	R/**H**	H	R	S	R	R
UX0771-2-99	R	R/**H**	S	H	H	R	H
UX0773-15-61	R	R/**H**	H	H	S	R	S
UX0792-6-1	R	R/**H**	H	H	R	R	S
UX0771-2-102	H	**R**/H	R	R	H	R	H
UX0771-2-104	H	**R**/H	H	H	H	R	S
UX0773-15-2	H	**R**/H	H	R	S	R	S
UX0773-15-56	H	**R**/H	H	H	R	R	H

Figures

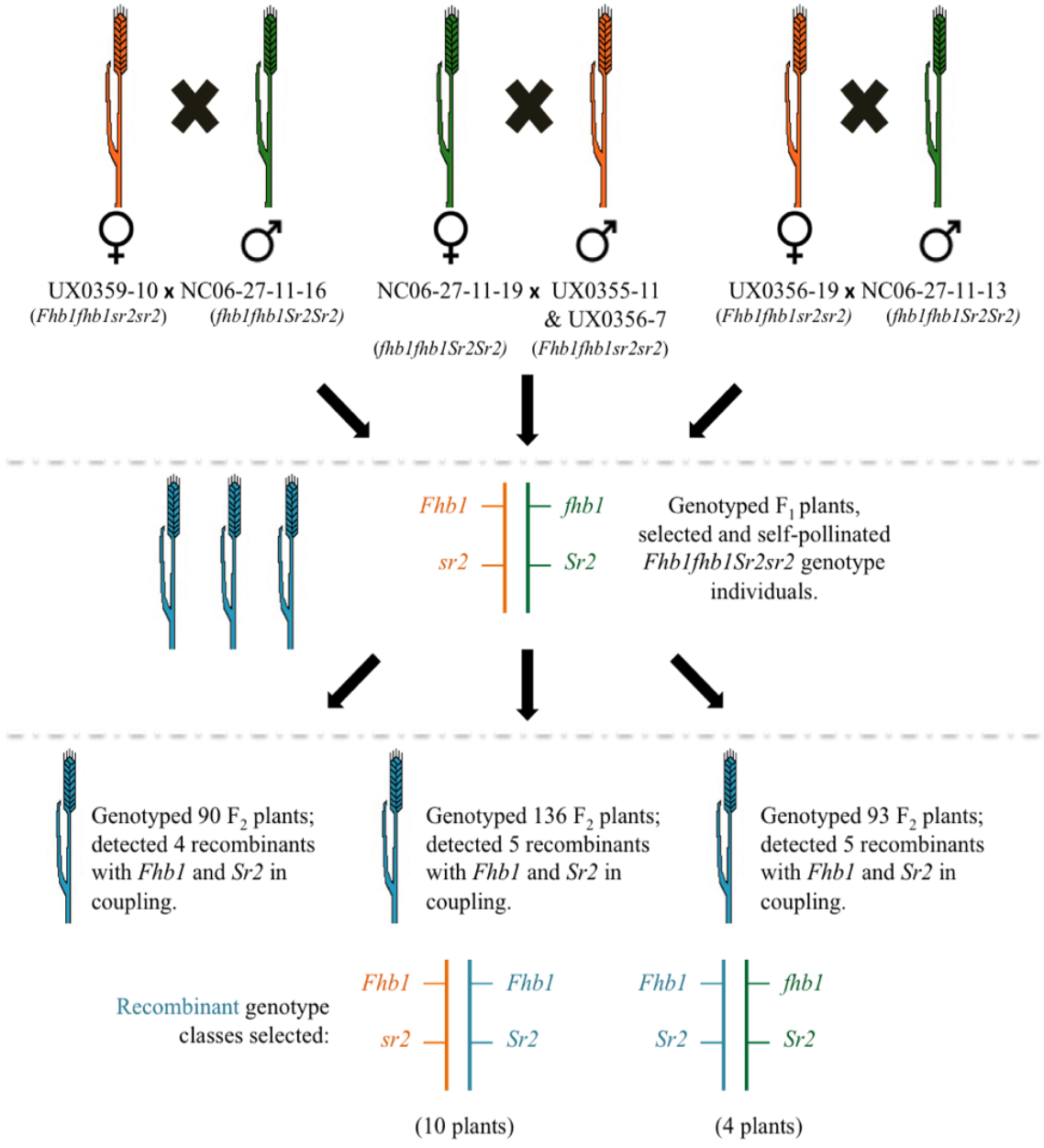
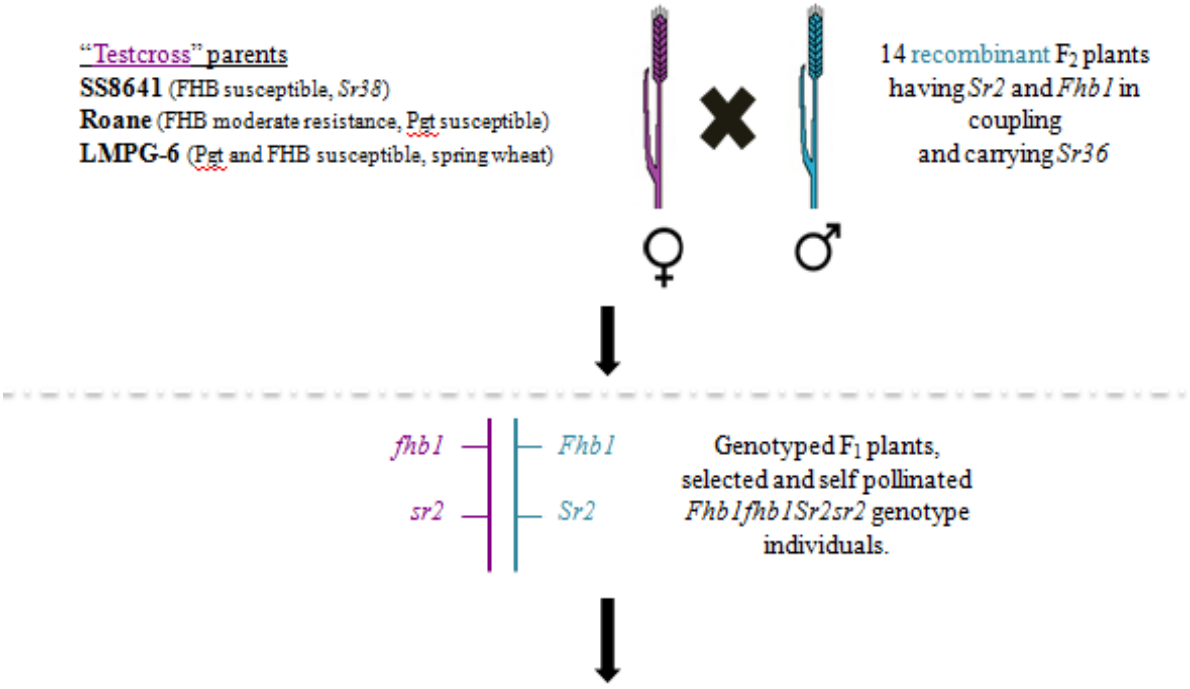


Figure 1. Crossing scheme for developing plants having *Fhb1* and *Sr2* in coupling phase. Fifty-four recombinant gametes were identified in the 319 F_2 plants; fourteen of the sixteen recombinants having *Fhb2* and *Sr2* in coupling were used for further crossing. Parental-type gametes are shown in green or orange font. Recombinant gametes with *Fhb1* and *Sr2* in coupling phase are shown in blue font. All selected F_2 plants also carry the *Sr36* stem rust resistance gene.



Female parent	# of F_2 seedlings genotyped	# of homozygous resistant (*Fhb1Fhb1Sr2Sr2*) selected F_2	# of homozygous susceptible (*fhb1fhb1sr2sr2*) selected F_2	# of $F_{2:3}$ seeds available
LMPG-6	632	22	17	5,412
Roane	559	13	13	10,463
SS8641	87	20	10	9,655

F_2 plants with *Fhb1Fhb1Sr2Sr2* and *fhb1fhb1sr2sr2* genotypes were selected and self-pollinated. LMPG-6 and Roane seedlings were *Pgt* screened to select against *Sr36*. SS8641 selections were screened with FHB.

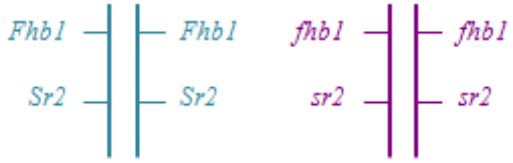
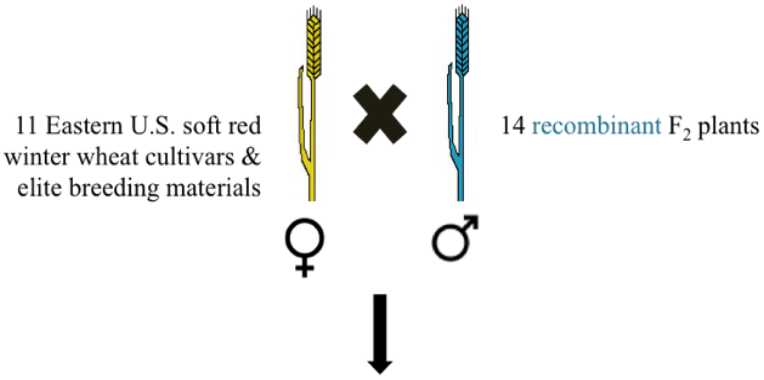


Figure 2. Crossing diagram for obtaining *Fhb1-Sr2* coupled gametes in *Pgt*- and *FHB*-susceptible backgrounds. Progeny will be screened for adult stem rust and FHB resistance, respectively, to confirm the presence of *Sr2* and *Fhb1*.



Female parent	# of Crosses	#of F_1 plants genotyped	# of F_1 plants selected	# of F_2 seeds available
AGS 2020	6	60	25	2,140
AGS 2035	4	51	11	839
AGS 2060	5	60	11	1,384
Caledonia R.	4	65	17	1,101
IL00-8530	1	17	6	432
IL00-8633	5	55	25	2,552
INW07031	2	25	9	674
McCormick	6	73	12	894
NC-Neuse	12	143	17	894
Shirley	5	73	19	2,172
VA01W205	1	10	1	93
Total	55	632	170	11,639

F_1 plants with *Fhb1fhb1Sr2sr2* genotype and at least three or more other resistance genes for pyramiding were selected and self-pollinated. Gametes:

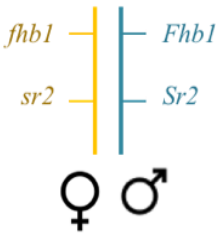
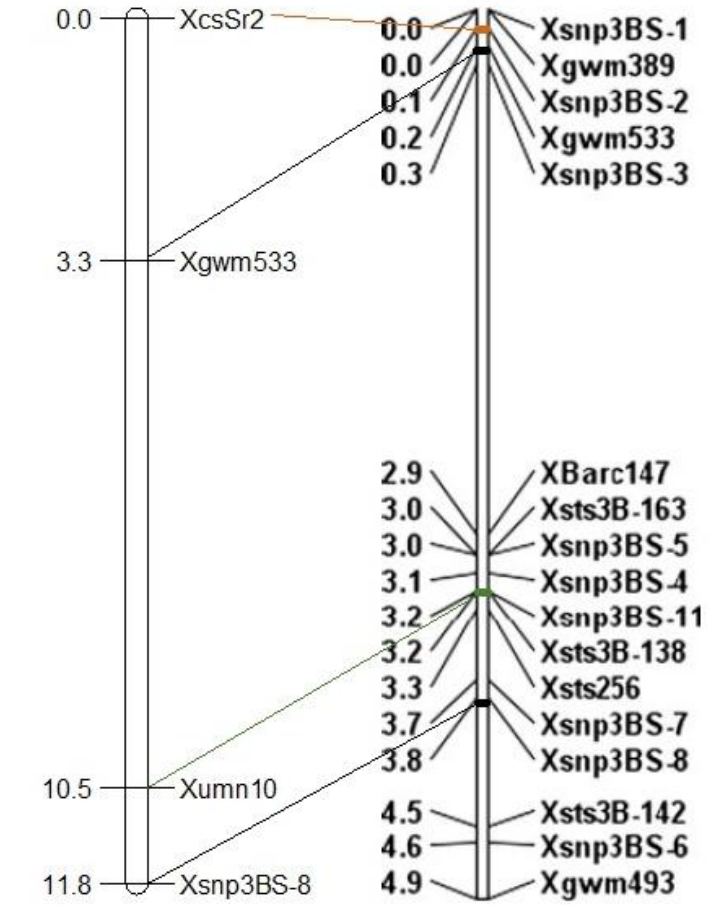


Figure 3. Crossing diagram for deploying *Fhb1* and *Sr2* in coupling in SWW genotypes of the eastern U.S. The 14 recombinants were crossed with 11 SWW cultivars for germplasm development.



4a. 4b.

Figure 4. Comparison of the authors' linkage map and a recently-published linkage map of the *Fhb1-Sr2* region of chromosome 3BS. 4a. Linkage map of chromosome 3BS for markers *Xsnp3BS-8*, *Xumn10*, *Xgwm533*, and *XcsSr2*, mapped in the UX07 F_2 population. Generated with JoinMap 3.0, using 319 population individuals (LOD=9.0). Numbers to the left of the vertical bar indicate the total distance in centimorgans (cM), mapped markers are on the right. 4b. Adaptation of the Bernardo et al. (2012) fine linkage map of the 3BS region with *Fhb1*, mapped in a Ning7840/Clark BC_7F_7 population. Numbers to the left of the vertical bar indicate the interval distance in cM, mapped markers are on the right. The lines across the figures represent the same genetic loci on the two maps. The green line represents the approximate location of *Fhb1*, the orange *Sr2*. Note: markers *Xumn10* and *Xsnp3BS-11* assay the same locus.

Figure 5. Example of pseudo-black chaff (PBC) in the F_2 plants. Photo, Emma Flemmig.

Figure 6. Example of variation in FHB infection spread on four heads from the same plant, inoculated on the same date. Photo, Emma Flemmig.

CHAPTER III:

Characterization and Origin of Chromosome 2B in Eastern Soft Winter Wheat Germplasm

Abstract

Introgressions from related species of bread wheat are an important source of disease resistance and quality traits in wheat breeding. One important introgression is the t2BS.2GS.2GL.2BL translocation, having the proximal regions of the long and short arms of chromosome 2G from *Triticum timopheevii* ssp. *timopheevii* translocated into wheat chromosome 2B. This translocation includes two important resistance genes—one for stem rust (*Sr36*) and the other for powdery mildew (*Pm6*). In recent studies of population structure in soft winter wheat, the presence of this translocation was found to distort estimates of genetic relationships among germplasm. With access to genotypic data for almost 600 SNP markers located on a new, high density map of chromosome 2B for 367 soft winter wheat (SWW) cultivars and advanced breeding lines from the eastern U.S, we were able to estimate of the size of the *T. timopheevii* introgression. Our data indicate that the alien introgression covers about 80% of the chromosome length and has reduced the diversity of haplotypes of 2B in the regional germplasm carrying the translocation. However, graphical genotypes of the SNPs indicate some diversity within lines carrying *Sr36* and suggest that two separate introgressions from *T. timopheevii* are present in eastern wheat germplasm. These two introgressions were associated with different alleles for photoperiod insensitivity at the *Ppd-B1* locus. We also determined that an allele reported to increase 1,000 grain weight is associated with the t2BS.2GS.2GL.2BL chromosome. These

data will provide eastern U.S. SWW breeders insight into the quality and resistance traits associated with in their *Sr36*-carrying germplasm in their programs. The large size and reduced recombination associated with the introgression will also provide insight into the difficulty of combining and introgressing new traits on 2B in lines having the translocated chromosome.

Introduction

Wheat stem rust, *Puccinia graminis* f. sp. *tritici* Pers. (*Pgt*), a devastating pathogen to wheat production, historically caused major epidemics in the United States during the early 20th century (McIntosh et al. 1995; Peterson 2001). Stem rust epidemics were managed well worldwide for almost 50 years with the use of host plant resistance, the removal of the causal agent's alternate host (*Berberis vulgaris* L.) common barberry (Peterson , 2001), and the cultivation of early maturing varieties (Marshall, 1989). *Sr31*, a stem rust resistance gene on the 1RS.1BL chromosome having the short arm of rye (*Secale cereale)* chromosome 1R translocated onto the long arm of wheat chromosome 1B, was widely-deployed around the world from the CIMMYT breeding programs (Singh et al., 2008). The 1RS.1BL introgression provided stem, leaf, and stripe rust and powdery mildew resistance.

There are many important introgressions from related species into wheat in addition to the 1RS.1BL translocation (Sharma et al., 1983; Jiang et al., 1994; Gill et al., 2006). Introgressions into bread wheat have been made from diploid, tetraploid, and hexaploid related species like wild and domesticated *Triticum* and *Aegilops* species (Sharma & Gill, 1983). In addition, methods have been developed to utilize the tertiary gene pool that includes *Agropyron, Hordeum, Elymus*, *Secale* and other species (Jiang et al., 1994). Many of these introgressions are an important source of disease resistance in bread wheat breeding programs. One widely used alien introgression into common wheat is of powdery mildew resistance gene *Pm6*, and stem rust resistance gene *Sr36* from *Triticum timopheevii* ssp. *timopheevii* (Allard & Shands, 1954; McIntosh, 1988; Jorgensen & Jensen, 1973).

The stem rust resistance gene *Sr36* was introgressed into hard red spring wheat germplasm: CI 12632 and CI 12633 (Allard and Shands 1954). *Sr36* is located on the t2BS.2GS.2GL.2BL translocation having the proximal regions of the long and short arms of chromosome 2G from from *Triticum timopheevii* ssp. *timopheevii* translocated into wheat chromosome 2B (Tsilo et al. 2008). *Sr36* is a widespread source of stem rust resistance in U.S. soft winter wheat (SWW) cultivars. It is found in approximately 20% of SWW cultivars, but is only present at a low frequency in other U.S. wheat market classes (Olsen et al. 2010). *Sr36* virulence has been detected in the U.S. as early as the 1940s (Y. Jin, personal communication), and in Canada since the early 1990s (Harder & Dunsmore, 1993). In the U.S., *Sr36* virulence is currently detected in the Pacific Northwest even though *Sr36* has never been released in that region (Y. Jin, personal communication). *Sr36* virulence was detected in Kenya in 2009 (Jin et al., 2009; FAO 2012).

Powdery mildew resistance gene *Pm6* is located in the long arm of the translocated chromosome, while *Sr36* is associated with the short arm (Tao et al. 2000). The size of this *T. timopheevii* introgression in the t2BS.2GS.2GL.2BL chromosome, is unknown but it is potentially large since it spans both chromosome arms (Tao et al., 2000). Since there is reduced pairing and recombination between 2G and 2B, the *Pm6* and *Sr36* genes are linked, but have occasionally been dissociated from each other (Jorgensen and Jensen 1973; Tao et al 2000). The introgression of *Sr36* from *T. timopheevii* into wheat germplasm CI 12633 included *Pm6* (Allard and Shands 1954), but there is also evidence of multiple *Pm6* introgressions into wheat (McIntosh and Gyarfas 1971; McIntosh and Luig 1973; Jorgensen

and Jensen 1973).

The t2BS.2GS.2GL.2BL chromosome is preferentially transmitted (Tsilo et al., 2008; Allard & Shands, 1954). In a population of 472 F_2 plants derived from the cross of an *Sr36* parent 'NC-Neuse' by an *Sr40* parent 'U5665-60,' extreme segregation distortion in favor of *Sr36* gametes was detected ($\chi 2 = 244.90$, $p<0.0001$) (Flemmig, unpublished data). It is very likely that this large introgression carries other genes from *T. timopheevii*. Black point is a damaging physiological response triggered in wheat during humid conditions at the end of the growing season. A black point resistance QTL has been associated with the t2BS.2GS.2GL.2BL translocation (Christopher et al., 2007; Lehmensiek et al., 2004). *Fusarium proliferatum*, and other various fungal species, have been associated with the degradation of the grain, but a clear link to a certain fungal species and black point symptoms have yet to be established (Conner et al. 1996; Lehmensiek et al., 2004; Christopher et al., 2007). While resistance to black point is a desirable aspect of the introgression, there are other traits located on chromosome 2B of wheat in the region of the translocation. For instance, QTL for milling and baking quality traits and for preharvest sprouting resistance were mapped to chromosome 2B in U.S. SWW (Campbell et al., 2001; Munkvold et al., 2009).

In addition, one of the major genes for photoperiod response in wheat, *Ppd-B1*, is located on the short arm of chromosome 2B. Two alleles conferring insensitivity to daylength have been identified at the *Ppd-B1* locus (Díaz et al., 2012). The alleles are described by the cultivar in which they were identified and allele specific markers are

available for screening germplasm. An allele found in the wheat line Chinese Spring conferring a daylength insensitive response has one truncated copy and three intact copies of a tandem repeat in the coding region. This copy number variant is thought to cause misexpression of the *Ppd-B1* gene, resulting in the daylength neutral phenotype. The allele specific marker for the copy number variant from Chinese Spring is named TaPpdBJ001. An additional gene for daylength insensitity is present in the cultivars Sonora 64. In this case, marker TaPpdBJ003, is dominant for an insensitive allele caused by tandem repeats in the coding region.

The sucrose synthase gene (*TaSus2-2B*) is also located on chromosome 2B (Jiang et al. 2011). Two haplotypes of the *TaSus2-2B* gene are associated with low and high 1,000 grain weight (TGW). Hap-H that is associated with high TGW has undergone strong positive selection in Chinese wheat breeding programs due to its association with higher yield (Jiang et al. 2011). *TaSus2-2B* was mapped to the same region of 2BS as the *T. timopheevii* introgression containing *Sr36*. A SNP marker is available for distinguishing between the Hap-H and Hap-L alleles (Jiang et al. 2011).

Recently the first massively parallel SNP genotyping assay was developed for *T. aestivum*. The wheat 9K iSelect assay marketed by Illumina, Inc. (San Diego, CA) has approximately 8,600 SNP markers. The development of these SNP markers provides wheat researchers with large quantities of genotyping data for a single DNA sample at a low cost per marker. The 9K iSelect assay is the first opportunity for wheat breeders to access whole genome SNP genotyping data. Fourteen eastern wheat breeders collaborated to have a total

of 558 samples of U.S. soft wheat germplasm genotyped with the 9K iSelect assay. The combination of genotypic data for a genome-wide collection of markers for hundreds of wheat lines and genotyping with allele specific markers for the *Sr36, TaSus2-2B*, and *Ppd-B1* traits on 2B will provide us with a picture of the size of the *T. timopheevii* introgression and its relationship with other traits.

Given the reduced recombination of the t2BS.2GS.2GL.2BL introgression with wheat chromosome 2B, preferential transmission of the alien segment, and widespread presence of the translocation, we expect to see an effect on the haplotype structure of chromosome 2B in eastern soft winter wheat germplasm. Our goal is to determine the effect of the large alien introgression on diversity of wheat chromosome 2B in wheat breeding programs in the eastern U.S.A.

Materials and Methods

Plant material

In total, the plant material used included 367 soft winter wheat cultivars and breeding lines obtained from 13 breeding and genetic programs in the eastern U.S. (Table 1). Genomic DNA was isolated at the USDA-ARS Eastern Regional Small Grains Genotyping Laboratory at Raleigh, North Carolina from 222 cultivars and breeding lines provided by eleven breeding programs. Seeds were germinated and two 2.5 cm segments of tissue were harvested from a single seedling in 96-well plates filled with silica gel at room temperature. After desiccation in silica was complete, tissue was macerated in the silica gel with a

GenoGrinder 2000 (SPEX CertiPrep, Metuchen, NJ). DNA extractions were performed according to a modified SDS protocol originally published in Pallotta et al. (2003). Genomic DNA of 137 cultivars was isolated at the Soft Wheat Quality Lab at Wooster, Ohio, and DNA of seven cultivars was provided by Dr. Mark Sorrells at Cornell University. Multiple single plant samples were isolated in the same manner from cultivars provided by the USDA Small Grains Collection in Aberdeen, Idaho. These plant materials included: PI 94761, one of the *T. timopheevii* accessions from which *Sr36* was transferred into *T. aestivum*; CI 15232, the *T. aestivum* accession that was crossed to PI 94761; CI 12633, one of two original lines with the *Sr36 T. timopheevii* introgression from the PI 94761/CI 15232 cross; and a collection of cultivars of interest from the pedigrees of many SWW lines in our population and germplasm from crosses with *T. timopheevii*. These lines were Timvera, Timstein, Steinwedel, Coker 65-20, and CI 14958.

Genotyping

DNA plates were sent for genotyping to the Cereal Crops unit of the USDA-ARS Northern Crop Science Research Laboratory in Fargo, North Dakota. The 367 cultivars were genotyped according to manufacturer's instructions using the Wheat 9K iSelect assay (Illumina, Inc., San Diego, CA). The Wheat 9K iSelect assay was developed by E. Akhunov and M. Hayden in collaboration with S. Chao, G. Brown-Guedira, M. Sorrells, E. See, and C. Cavanagh. SNP markers from the Infinium assay were analyzed using GenomeStudio V2010.3 software (Illumina, Inc., San Diego, CA) by personnel of the USDA-ARS Eastern

Regional Small Grains Genotyping Laboratory at Raleigh, NC. Additional single-nucleotide polymorphism (SNP), and simple sequence repeat (SSR) markers on chromosome 2B were used for genotyping. Markers used were the SSR marker *Xwmc477* linked to *Sr36* (Tsilo et al. 2008), the *TaSus2-2B*_1 KASPar assay that distinguishes Hap-H and Hap-L alleles at *XTaSus2-2B* (Jiang et al., 2011), and two KASPar assays for alleles at the *Ppd-B1* locus: TaPpdBJ001 and TaPpdBJ003 (Díaz et al., 2012).

Polymerase chain reaction (PCR) amplification of wmc477 was performed in a 12 µL reaction volume consisting of 2 µL (20 ngμL^{-1}) genomic DNA template, 1.20 µl 10X PCR buffer (10 mM Tris-HCL, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 0.96 µl 2.5 mM mixture of dNTPs, 0.30 µl forward primer (5 µM), 0.30 µl reverse primer, 0.09 µl of *Taq* polymerase, and 7.15 µl of water, bringing the reaction total to 12. The forward primer was labeled with 6-FAM fluorescent dye. Thermal cycler PCR conditions were: (i) initial denaturation at 95°C for 2 min; (ii) 35 amplification cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C; (iii) 5 min final extension at 72°C; (iv) 4°C hold. Amplifications were performed using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Separation of fragments was performed by capillary electrophoresis using an ABI3130xl Genetic Analyzer (Applied BioSystems, Foster City, CA). Data were analyzed using GeneMarker 1.85 software (SoftGenetics, State College, PA).

KASPar assays for *TaSus2-2B* (Jiang et al. 2011), TaPpdBJ001 and TaPpdBJ003 (Díaz et al. 2012) (Table 1) were performed according to manufacturer's instructions (KBioscience Ltd., Hoddeson, UK). A Roche Lightcycler® 480 Real-Time PCR instrument

and software were used for endpoint genotyping (F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Data analysis

The consensus SNP genetic map developed by C. Cavanagh and E. Huang was provided prior to publication. The genotyping data for map construction was provided by C. Cavanagh, E. Akhunov, J. Dubcovsky, S. Chao, and A. Carter. The map estimated the size of chromosome 2B as almost 400 cM. This is an over-estimate of the actual genetic distance of chromosome 2B. The genetic distances are larger than expected because of the method, termed the MAGIC population for multiple advanced generation intercross, that was used to create a population for mapping the SNP undergoes more generations of recombination. Eight cultivars were intercrossed and underwent multiple rounds of recombination prior to self-pollinating, which exaggerates the distance between the markers but not the marker order. This map was utilized with our genotyping data to estimate the size of the t2BS.2GS.2GL.2BL translocation.

In order to determine differences in allelic diversity on chromosome 2B in lines with and without the translocation and over time, Polymorphism Information Content (PIC) values were calculated for all 579 markers on 2B using the formula:

$$PIC = 1 - \sum_{n=1}^{i} (p^2 + q^2 + n^2)$$

where *p* equals the frequency of AA genotypes, *q* equals the frequency of BB genotypes,

and *n* equals the frequency of null genotypes. The 579 markers were the 576 SNPs from the 9K SNP chip, *TaSus2-2B,* and the two photoperiod markers. Cultivars were divided into five groups for comparison of mean PIC values to determine the effect of the *T. timopheevii* introgression on diversity in cultivars with and without the translocation. The subsets included: (1) all cultivars, (2) only cultivars released before 1950 (the original report of the *Sr36*-introgression was in 1954), (3) only cultivars released after 1950, (4) only cultivars without *Sr36,* and (5) only cultivars with *Sr36* (all released after 1950). In addition, mean PIC values were calculated for the cultivar subsets using markers from different chromosomal regions to determine the affect of the translocation on diversity in the flanking regions of the chromosome. The chromosomal regions were: (1) all markers along the length of the chromosome (0.00 to 395.92 cM), (2) markers on 2BS distal to the 2G ingtrogression (0 to 43.76 cM), (3) markers on distal on 2BS in a region that may or may not include the translocation (44.81 to 102.25 cM), (4) 49 translocation-specific markers, (5) all markers in the region of the translocation (104.63 to 303.68 cM), and (6) markers on 2BL distal to the translocation (304.01 to 395.92 cM).

Graphical genotypes

To illustrate the parentage of the alleles in SWW germplasm, an excel file of SNP genptypes were color-coded to according to the presence of alleles in founding and modern cultivars. Initially, the cultivars' alleles in common with 'Mediterranean,' an important founding cultivar of SWW germplasm and a landrace brought to the U.S. from Europe,

were color-coded dark green. Alleles not previously colored green and in common with the founder ‘Purplestraw’ were then color-coded purple. We continued to color-code the file in a step-wise fashion based on allele sharing with founding cultivars in the following order: ‘Mediterranean,’ collected in 1837 (dark green); ‘Purplestraw,’ 1822 (purple); ‘Fultz,’ 1871 (golden yellow); ‘Genesee Giant,’ 1893 (red); ‘Harvest Queen,’ 1897 (light yellow); ‘Grandprize,’ 1904 (light green). Two modern cultivars were also used in the color-coding: NC-Neuse, released in 2003 (blue); and AGS 2000, released in 2002 (gray). Alleles specific to the *T. timopheevii* translocation were colored orange. While these graphical genotypes represent allele sharing rather than identity by descent, they provide a graphical representation of the recombination and shared haplotypes along the chromosome in diverse germplasm.

Results

Genotyping major genes

The 185 bp fragment of the SSR marker *Xwmc477* is tightly linked with the resistance gene *Sr36* (Tsilo et el., 2008). This fragment was observed in the *T. timopheevii* wheat parent (PI 94761) of the t2BS.2GS.2GL.2BL introgression into the common wheat germplasm CI 12633 containing *Sr36* and *Pm6*. Two selected lines that involve different crosses with *T. timopheevii*, CI 14958 (pedigree: *T. timopheevii*/Shands' 473//Cheyenne/3/Pawnee/ Cheyenne), and Timvera (pedigree: Steinwedel/*T. timopheevii*), also amplified the 185 bp allele. Among the SWW breeding lines and cultivars tested, 90 out of 367 have the *T.*

timopheevii-derived allele at *Xwmc477* (Table 1).

A nearly identical segregation pattern was observed when cultivars were evaluated with a KASP assay for the SNP in the *TaSus2-2B* locus. The Hap-H allele was not observed in any eastern wheat lines released prior to 1950 and were present only at a low frequency in lines not having the *T. timopheevii* introgression (Table 3). Of the eastern wheat lines, there were 97 with a Hap-H genotype (Table 1). The 97 Hap-H cultivars and breeding lines aligned perfectly with the *Sr36* positive germplasm except ARS05-0401, ARS05-0441, Clark, IL07-4415, INW0731, INW1021, P03528A1-10, and VA01W-476, which have the Hap-H allele and lack the 185 bp allele of *Xwmc477* associated with *Sr36*.

Two *Ppd-B1* assays were used to detect the insensitivity alleles from 'Chinese Spring' (CS) and 'Sonora 64.' When assayed for alleles at the *Ppd-B1* locus using marker TaPpdBJ001, 56 contemporary eastern wheat lines along with 'Timstein,' 'Coker 65-20,' 'Steinwedel,' and CI 12633 carried the insensitive allele present in CS that is associated with the three intact and one truncated copy of the gene (Table 1). A distinctive pattern was observed for marker TaPpdBJ001 among lines. The CS insensitive allele was detected in approximately half of the lines having the 185 bp fragment of *Xwmc477* while the sensitive allele predominates in cultivars without the translocation (Tables 1 and 3). This photoperiod insensitivity gene was not present in any eastern wheat cultivars developed before 1950 (Table 3). Thirteen contemporary eastern wheat lines (ARS05-0401, ARS05-0441, Chesapeake, IL00-8530, IL00-8633, IL06-13721, IL06-14262, IL97-6755, NC08-23089, NC08-23090, P07469A1-28, and Pioneer 25R18.), as well as Coker 65-20, Steinwedel, and

Timstein have the CS insensitive allele and do not have the translocation. The other half of the lines tested that carry the introgression from *T. timopheevii* did not amplify a product with the KASP assay TaPpdBJ001 and were considered to have a null allele. This null allele was exclusively observed in lines having the translocation (Tables 1 and 3). The CS null allele was not observed in any lines that did not have the 185 bp allele of *Xwmc477* (Tables 1 and 3).

There are two common haplotypes in cultivars having the *T. timopheevii* introgression. Forty-four lines and CI 12633 have the *Xwmc477* allele for *Sr36*, high TGW allele at *TaSus2-2B*, and the *Ppd-B1* CS insensitive allele (Tables 1 and 3). The other 41 lines, CI 14958, Timvera, and PI 94761 have the *Xwmc477* alleles for *Sr36*, high TGW allele at *TaSus2-2B*, and a previously unreported null allele for *Ppd-B1* (Tables 1 and 3). There are four lines that have *Sr36* and Hap-H, but have the CS photoperiod sensitive allele of *Ppd-B1*: Adder, Ernie, MD99W64-05-11, and MO 050921.

An interesting pattern was observed with the marker for the insensitive allele at *Ppd-B1* locus that was described in Sonora 64 (Diaz et al. 2012). Amplification with KASPar marker TaPpdBJ003 was rare in eastern wheat germplasm (Tables 1 and 3). Only 20 lines, almost exclusively developed by breeding programs in Georgia and Louisiana, have this allele for photoperiod insensitivity (Table 1). These cultivars all lack *Sr36* and are relatively recently developed lines.

SNP genotyping

Based on chromosome locations provided by Cavanaugh and Huang (personal communication), 576 SNP markers were polymorphic in the set of 376 eastern wheat lines genotyped were located on chromosome 2B, covering a distance of almost 400 cM on the consensus map (Supplementary Table 2). We detected 49 SNP markers that co-segregated with the 185 bp allele of *Xwmc477* and thus appear to be specific to the *Sr36* introgression. Of the 49 introgression-specific markers, 17 produced null alleles in lines having the *T. timopheevii* introgression. The other 32 markers have SNP alleles specific to the alien introgression that are not null alleles but are monomorphic in lines without the translocation. Our data indicated that 436 SNP markers lie between the most distal and proximal *T. timopheevii* introgression-specific markers. For 53 markers located immediately distal to the SNP specific to the translocation on 2BS, two distinct haplotype blocks were detected (Figure 1), and it is not certain if these markers are located on in the region of the introgression. According to the consensus map positions, our estimate is that the original introgression covered a region of at least 200 cM.

When graphical genotypes of chromosome 2B in eastern U.S. SWW germplasm were visualized, a large haplotype block representing the t2BS.2GS.2GL.2BL translocation is easily seen (Figure 1). These graphical genotypes indicate that in lines having the alien introgression, the portion of the t2BS.2GS.2GL.2BL chromosome derived from *T. timopheevii* stretches almost of the entire length of the chromosome with only the most distal regions of the long and short arms being derived from *T. aestivum*. In lines carrying

the introgression, most of the chromosome is fixed and historical recombination events are not observed.

When lines carrying the translocation are divided into those having different alleles at *Ppd-B1* (CS insensitive and CS null), the two haplotype blocks consisting of 53 SNP markers spanning 60 cM on the short arm can be seen. Since no *T. timopheevii* specific SNP markers are present in this region, we cannot determine if these SNP are derived from 2B or 2G. However, these haplotype blocks also suggest that the introgression containing *Sr36* in eastern germplasm may have been derived from different sources of the translocation.

Diversity of lines

The PIC value of every marker was calculated for six subsets based on the presence of *Sr36* and the age of the cultivars' release (Supplemental Data 1). Mean PIC values were calculated in 36 groups for marker diversity—by the presence or absence of *Sr36*, date of release of the cultivar, and chromosomal region where marker lies (Table 2).

The overall mean PIC for all markers along the chromosome in the five groups of cultivars ranged from 0.07 to 0.37. The mean PIC of all SNP for lines released prior to 1950 was 0.28 compared to 0.37 for all lines released after 1950, indicating that marker diversity for chromosome 2B has increased over time. Mean PIC value of 0.07 for lines having the translocation indicate that these were the least diverse along the length of the chromosome. However, when different regions of the chromosome are considered, this difference is less pronounced. The mean PIC of all markers from 2G on the t2BS.2GS.2GL.2BL translocation

was zero since the markers were monomorphic in lines having the translocation (Table 2).

The mean PIC values in the distal region of 2BS were only slightly less for lines having the translocation (0.36) compared to cultivars without (0.40). Differences in the distal portion of the long arm were greater, with mean PIC of 0.21 in lines having *Sr36* and 0.36 in lines without *Sr36*.

Discussion

Beginning as an initiative to link stem rust resistance genes *Sr36* and *Sr40* that are both located on chromosome 2B with the assistance of molecular markers, we found that the segregation distortion favoring selection of the *T. timopheevii* introgression was so large that we could not recover recombinant gametes from heterozygous individuals (data not shown). Recognizing this distortion led to inquiries about the general effects of the using the *Sr36* introgression in breeding programs. It seemed fair to assume that the introgression is unintentionally selected as frequently as it is intentionally selected in breeding programs since stem rust infection is not usually observed in eastern wheat breeding nurseries. We wanted to determine if the favorable distortion for *Sr36* selection was preventing breeders from selecting for other genes. Due to its segregation distortion, we wanted to determine if breeders would have enough germplasm diversity to utilize if an *Sr36*-virulent race establishes in the eastern United States and get an impression of the difficulty for the removal of *Sr36* in eastern SWW breeding materials.

The presence of the translocation in eastern germplasm has a large effect on genetic diversity of lines. Previous studies in our lab have determined that the presence/absence of t2BS.2GS.2GL.2BL chromosome alters the population substructure of eastern SWW even when the entire *T. aestivum* genome is considered (Benson and Merrill, unpublished data). The large number of markers derived from chromosome 2G that are inherited as a block are weighted heavily in analysis of population structure such as principal component analysis.

When we examined marker diversity on chromosome 2B, we found that cultivars released before 1950 were the least diverse, except for lines having the translocation. Allelic diversity has increased along chromosome 2B since the 1950s where *Sr36* is not present. The complete lack of allelic diversity on the translocation in cultivars carrying it supports recombination suppression and shows that increased selection for *Sr36* would reduce diversity in eastern SWW germplasm. Interestingly, when only the distal regions of chromosome 2B are considered, *Sr36* positive cultivars are almost as diverse as the other cultivars for markers in the short arm. But there appears to be a reduction in diversity in the distal part of the long arm.

Except in eight lines, U.S. SWW breeders have not introgressed the Hap-H allele of *TaSus2-2B* into their germplasm without *Sr36*. Overall, the introgression seems to carry entirely positive traits from *T. timopheevii* – black point resistance, increased milling quality genes, powdery mildew and stem rust resistance. However, our data indicate that it will be difficult to combine this alien introgression with an allele for photoperiod sensitivity (the Sonora 64 allele) present in lines developed in Georgia and Louisiana.

We determined that there are differences in alleles at the *Ppd-B1* locus in eastern lines having *Sr36.* PI 94761 (*T. timopheevii*) and CI 12633 (the original *T. aestivum* line with *Sr36*) do not have the same genotype with the TaPpdBJ001 marker. This suggests that the *Ppd-B1* gene in CI 12633 was not derived from *T. timopheevii*. However, the newly detected null allele for this marker was only observed in lines having the *T. timopheevii* introgression, including Timvera, as well as in the *T. timopheevii* accession PI 94761. It is possible that this allele was derived from *T. timopheevii* and introduced into eastern wheat germplasm through 'Timvera.'

Having multiple, previously-unidentified introgressions of *Sr3*6 into U.S. germplasm, may help us work with combining other alleles in the region. Our access to many markers associated with the introgression(s) and traits identified in other studies on 2B could facilitate an improved understanding of the traits available on different introgressions. The photoperiod allele differences may also dictate the preferred source of the introgression in some regions.

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Tables

Table 1. Genotypes of *TaSus2-2B, Ppd-B1*alleles, and *Sr36* for all cultivars and advanced breeding lines. *TaSus2-2B* is the gene *Sucrose Synthase2*. L and H indicate low and high TGW alleles, respectively. TaPpdBJ001 and TaPpdBJ003 are the markers for 'Chinese Spring' and 'Sonora 64' photoperiod alleles, respectively, assaying different alleles at locus *Ppd-B1*. S and I indicate photoperiod sensitivity and insensitivity alleles, respectively. *Sr36* was identified as absent and present with *Xwmc477*. NA indicates missing data; Het, a heterozygous genotype; and null, a negative allele. The program abbreviations represent the states where the line originated. The '-' indicates a recent breeding lines or an unregistered or protected cultivar.

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
001169-7E15	GA	-	L	S	null	Absent
01063-1-3-6-2-G2	GA	-	L	S	null	Absent
011124-1-42-13	GA	-	L	S	null	Absent
011388-8-4-5	GA	-	L	S	null	Absent
031086-44-4-2	GA	-	L	S	null	Absent
051336-B-B-1	GA	-	L	S	null	Absent
071628-G3-G1-G4-G1	GA	-	L	S	I	Absent
081515-G1-G2	GA	-	L	S	I	Absent
09283-G1-G1	GA	-	L	S	I	Absent
991227-6A33	GA	-	L	S	I	Absent
991371-6E12	GA	-	L	S	I	Absent
Abe	IN	**1972**	H	I	null	Present
Adder	IN	**1986**	H	S	null	Present
Adena	OH	**1985**	H	null	null	Present
AGS 2020	GA	**2002**	L	S	null	Absent
AGS 2000	GA	**2002**	L	S	I	Absent
AGS 2010	GA	**2006**	L	S	I	Absent
AGS 2020	GA	**2008**	L	S	I	Absent
AGS 2026	GA	**2010**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
AGS 2031	GA	**2006**	H	null	null	Present
AGS 2035	GA		NA	NA	NA	Absent
AGS 2060	GA	**2011**	H	I	null	Present
AGS 2485	GA		L	S	null	Absent
AGS CL7	GA		L	S	I	Absent
Allegiance	KY	**2002**	L	S	null	Absent
American Banner	MI	**1922/1881**	L	S	null	Absent
Argee	WI	**1976**	L	S	null	Absent
ARS05-0074	ARS-NC	-	H	I	null	Present
ARS05-0282	ARS-NC	-	H	I	null	Present
ARS05-0401	ARS-NC	-	H	I	null	Absent
ARS05-0441	ARS-NC	-	H	I	null	Absent
ARS07-0046	ARS-NC	-	H	I	null	Present
ARS07-0154	ARS-NC	-	L	S	null	Absent
ARS07-0203	ARS-NC	-	H	null	null	Present
ARS07-0404	ARS-NC	-	H	null	null	Present
ARS07-0525	ARS-NC	-	H	I	null	Present
ARS07-0558	ARS-NC	-	H	I	null	Present
ARS07-0815	ARS-NC	-	H	I	null	Present
ARS07-0912	ARS-NC	-	H	I	null	Present
ARS07-1159	ARS-NC	-	H	I	null	Present
ARS07-1208	ARS-NC	-	L	S	null	Absent
ARS07-1243	ARS-NC	-	L	S	null	Absent
ARS08-0111	ARS-NC	-	H	null	null	Present
ARS09-435	ARS-NC	-	H	I	null	Present

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
ARS09-776	ARS-NC	**-**	H	I	null	Present
Arthur	IN	**1974**	H	null	null	Present
Arthur_71	IN	**1974**	H	I	null	Present
Augusta	MI	**1986**	L	S	null	Absent
Baldwin	GA	**1994**	L	S	I	Absent
Batavia	NY	**1996**	L	S	null	Absent
Becker	OH	**1988**	L	S	null	Absent
Benhur	IN	**1978**	L	S	null	Absent
Blazer	CO	**1984**	L	S	null	Absent
Blueboy	NC	**1967**	L	S	null	Absent
Boone	IN	**1993**	L	S	null	Absent
Bradford	TX	**1985**	L	S	null	Absent
Brandy		**1988**	L	S	null	Absent
Branson		**2005**	L	S	null	Absent
Caldwell	IN	**1982**	L	S	null	Absent
Caledonia	NY	**2004**	L	S	null	Absent
Cardinal	OH	**1988**	L	S	null	Absent
Cayuga	NY	**1998**	L	S	null	Absent
Chancellor	GA	**1958**	L	S	null	Absent
Charmany	WI	**1984**	L	S	null	Absent
Chelsea	Canada	**pre-1950**	L	S	null	Absent
Chesapeake	MD	**2007**	L	I	null	Absent
CI 12633	WI	**1954**	H	I	null	Present
CI 14958	NE	**1963**	H	null	null	Present

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
CI 15232	WI	**1954**	L	S	null	Absent
Clark	IN	**1988**	H	S	null	Absent
Clemens	IN	**-**	L	S	null	Absent
Clemson 201	SC	**1996**	L	S	null	Absent
Coker 47-27	SC	**1950**	H	I	null	Present
Coker 65-20		**1967**	L	S	null	Absent
Coker 68-15	SC	**1971 / 1981**	L	S	null	Absent
Coker 747	SC	**-**	H	I	null	Present
Coker 762	SC	**1980**	H	null	null	Present
Coker 797	SC	**1980**	L	S	null	Absent
Coker 833	SC	**1984**	H	null	null	Present
Coker 9134	AR	**1992**	L	S	null	Absent
Coker 9152	AR	**2002**	null	S	null	Absent
Coker 916	SC	**1980**	H	null	null	Present
Coker 9375	AR	**2004**	L	S	null	Absent
Coker 9436	AR	**2005**	H	I	null	Present
Coker 9553	AR	**-**	L	S	null	Absent
Coker 9663	AR	**1997**	H	I	null	Present
Coker 9766	SC	**1987**	H	null	null	Present
Coker 9803	AR	**1990**	H	I	null	Present
Coker 983	SC	**-**	H	null	null	Present
Coker 9835	AR	**-**	H	null	null	Present
Compton	IN	**1983**	H	I	null	Present
Cornell 595	NY	**1942**	L	S	null	Absent
Delaware		**1992**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Delta King (GR9108)		**-**	L	null	null	Absent
Delta Queen	SC	**1978**	L	S	null	Absent
Dominion		**-**	H	null	null	Present
Doublecrop	AR	**1975**	H	I	null	Present
Dynasty	OH	**1987**	L	S	null	Absent
Elkhart	IN	**1996**	L	S	null	Absent
Ernie	MO	**1994**	H	S	null	Present
Excel	OH	**1990**	L	S	null	Absent
Fairfield	IN	**1942**	L	S	null	Absent
FFR 544W	IN	**1992**	L	S	null	Absent
FFR 555W	IN	**1991**	H	I	null	Present
FG95195		**-**	L	S	I	Absent
FL 302	FL	**1985**	L	S	null	Absent
Flint	GA	**1919**	L	S	null	Absent
Foster-AM65	KY	**-**	H	I	null	Present
Frankenmuth	MI	**1979**	L	S	null	Absent
Freedom	OH	**1991**	H	null	null	Present
Fulcaster	MD	**1913**	L	S	null	Absent
Fultz	PA	**1871**	NA	NA	NA	Absent
GA00067-8E35	GA	**-**	L	S	null	Absent
GA001138-8E36	GA	**-**	L	S	null	Absent
GA001142-9E23	GA	**-**	L	S	null	Absent
GA001170-7E26	GA	**-**	L	S	null	Absent
GA011493-8E18	GA	**-**	L	S	I	Absent
GA021245-9E16	GA	**-**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
GA021338-9E15	GA	**-**	L	S	null	Absent
GA031238-7E34	GA	**-**	H	null	null	Present
Genesee	NY	**1950**	L	S	null	Absent
Genesee Giant		**1893**	L	S	null	Absent
Glacier	WI	**1991**	L	S	null	Absent
Glory	OH	**1992**	L	S	null	Absent
Goens	IN	**1808**	L	S	null	Absent
Gore	GA	**1990**	L	S	null	Absent
GR 942		**1994**	L	S	null	Absent
Grandprize	NY	**1904**	L	S	null	Absent
Hart	MO	**1976**	L	S	null	Absent
Harus		**1985**	null	null	null	Absent
Harvest Queen	KS	**1897**	L	S	null	Absent
Hickory	IN	**1993**	L	S	null	Absent
Hillsdale	MI	**1983**	L	S	null	Absent
Holley	GA	**1970**	L	S	null	Absent
Honor	NY	**1920**	L	S	null	Absent
Hopewell	OH	**1995**	L	S	I	Absent
Hopkins	NY	**2009**	NA	NA	NA	Absent
Houser	NY	**1977**	L	S	null	Absent
Howell	IL	**1991**	L	S	null	Absent
Hunter	SC	**1982**	H	null	null	Present
IL00-8061	IL	**-**	L	S	null	Absent
IL00-8530	IL	**-**	L	I	null	Absent
IL00-8633	IL	**-**	L	I	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
IL00-8641	IL	-	L	S	null	Absent
IL01-34159	IL	-	L	S	null	Absent
IL02-18228	IL	-	L	S	null	Absent
IL05-4236	IL	-	L	S	null	Absent
IL06-13721	IL	-	L	I	null	Absent
IL06-14262	IL	-	L	I	null	Absent
IL06-23571	IL	-	L	S	null	Absent
IL07-19062	IL	-	L	S	null	Absent
IL07-20728	IL	-	L	Het	null	Absent
IL07-24841	IL	-	L	S	I	Absent
IL07-4415	IL	-	H	S	null	Absent
IL08-24578	IL	-	L	S	null	Absent
IL08-8844	IL	-	L	S	null	Absent
IL96-6472	IL	-	L	S	null	Absent
IL97-1828	IL	-	L	S	null	Absent
IL97-6755	IL	-	L	I	null	Absent
Illini Chief	IL	**1915**	L	S	null	Absent
INW 0101	IN	**2001**	L	S	null	Absent
INW 0303	IN	**2003**	L	null	null	Absent
INW 0411	IN	**2005**	H	I	null	Present
INW0304	IN	-	H	null	null	Present
INW0412	IN	-	L	S	null	Absent
INW0731	IN	-	H	S	null	Absent
INW1021	IN	-	H	S	null	Absent
Ionia	MI	**1969**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Jackson	VA	**1993**	L	S	null	Absent
Jamestown	VA	**2007**	L	S	null	Absent
Jaypee	AR	**1995**	H	I	null	Present
Jensen	NY	**2008**	NA	NA	NA	Absent
Kanqueen	KS	**1975**	NA	NA	NA	Absent
Kaskaskia	IL	**1998**	L	S	null	Absent
Key	IN	**1976**	L	S	null	Absent
Knox 62	IN	**1962**	L	S	null	Absent
Kristy		**2001**	L	S	null	Absent
KY02C-1002-06	KY	-	H	I	null	Present
KY02C-1043-04	KY	-	L	S	null	Absent
KY02C-1058-03	KY	-	L	S	null	Absent
KY02C-1076-07	KY	-	L	S	null	Absent
KY02C-1121-11	KY	-	L	S	null	Absent
KY02C-2215-02	KY	-	L	S	null	Absent
KY03C-1002-02	KY	-	L	S	null	Absent
KY03C-1237-39	KY	-	L	S	null	Absent
KY93C-1238-17-1	KY	-	L	S	null	Absent
KY94C-0094-11-2	KY	-	H	null	null	Present
KY97C-0321-05-2	KY	-	L	S	null	Absent
KY97C-0519-04-05	KY	-	L	S	null	Absent
LA01069D-23-4-4	LA	-	L	S	null	Absent
LA0110D-150	LA	-	L	S	null	Absent
LA01139D-56-1	LA	-	H	I	null	Present
LA01164D-94-2-B	LA	-	H	I	null	Present

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
LA02015E201	LA	-	L	S	null	Absent
LA02015E42	LA	-	L	S	null	Absent
LA02015E58	LA	-	L	S	null	Absent
LA02024E12	LA	-	L	S	null	Absent
LA02024E7	LA	-	L	S	null	Absent
LA03012E-27	LA	-	L	S	null	Absent
LA03118E117	LA	-	H	null	null	Present
LA03136E71	LA	-	L	S	I	Absent
LA03148E12	LA	-	H	I	null	Present
LA03155D-P13	LA	-	L	S	I	Absent
LA03161D-P1	LA	-	H	I	null	Present
LA03217D-P2	LA	-	L	S	null	Absent
LA03217E2	LA	-	L	S	null	Absent
LA04013D-142	LA	-	L	S	null	Absent
LA04041D-10	LA	-	L	S	I	Absent
LA841	LA	-	L	S	null	Absent
LA95135	LA	-	L	S	null	Absent
LA97113UC-124	LA	-	L	S	null	Absent
Lewis	MO	**1967**	L	S	I	Absent
Logan	OH	**1968**	L	S	null	Absent
Longberry No. 1	NY	**1898**	L	S	null	Absent
Madison	VA	**1990**	H	I	null	Present
MAGNOLIA	MO	**2006**	L	S	null	Absent
Magnum	CO	**1983**	H	I	null	Present
Mallard	CO	**1991**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Massey	VA	**1981**	H	null	null	Present
McCormick	VA	**2002**	L	S	null	Absent
McNair 1813	NC	**1975**	H	I	null	Present
McNair 701	NC	**1972**	L	S	null	Absent
MD00W16-07-3	MD	**-**	L	S	null	Absent
MD01W215-05-10	MD	**-**	L	S	null	Absent
MD01W233-06-1	MD	**-**	L	S	null	Absent
MD01W28-08-11	MD	**-**	L	S	I	Absent
MD99W460-06-31	MD	**-**	H	I	null	Present
MD99W64-05-11	MD	**-**	H	S	null	Present
Mediterranean	EU	**1837**	L	S	null	Absent
Merl	VA	**2009**	L	S	null	Absent
Milton	MO	**2009**	L	S	null	Absent
Mitchell		**2001**	L	S	null	Absent
MO 011126	MO	**-**	L	S	null	Absent
MO 050921	MO	**-**	H	S	null	Present
MO 080104	MO	**-**	L	S	null	Absent
MO 081652	MO	**-**	L	S	null	Absent
MO 94-317	MO	**-**	L	S	null	Absent
MO 980525	MO	**-**	L	S	null	Absent
MO 980829	MO	**-**	L	S	null	Absent
Moking	KS	**1946**	L	S	null	Absent
Monon	IN	**1949**	L	S	null	Absent
Mountain		**1999**	L	S	null	Absent
MPV 57		**2005**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Nabob	OH	**1928**	L	S	null	Absent
NC06-19896	NC	**-**	H	null	null	Present
NC06-20401	NC	**-**	H	null	null	Present
NC07-20850	NC	**-**	H	null	null	Present
NC07-22432	NC	**-**	H	null	null	Present
NC07-23880	NC	**-**	H	null	null	Present
NC07-24445	NC	**-**	H	null	null	Present
NC07-25169	NC	**-**	H	null	null	Present
NC08-21273	NC	**-**	H	I	null	Present
NC08-23089	NC	**-**	L	I	null	Absent
NC08-23090	NC	**-**	L	I	null	Absent
NC08-23323	NC	**-**	L	S	null	Absent
NC08-23324	NC	**-**	L	S	null	Absent
NC08-23383	NC	**-**	L	S	null	Absent
NC-Cape Fear	NC	**1990**	H	null	null	Present
NC-Neuse	NC	**2003**	H	null	null	Present
NC-Yadkin	NC	**2010**	H	I	null	Present
Nittany	PA	**1918**	L	S	null	Absent
NY6432-10	NY	**1988**	L	S	null	Absent
NY87048W-7388	NY	**-**	NA	NA	NA	Absent
NY91017-8080	NY	**-**	NA	NA	NA	Absent
Oasis	IN	**1973**	L	S	null	Absent
Oglethorpe	GA	**2009**	L	S	null	Absent
P0175A1-31	PUR	**-**	H	null	null	Present
P03207A1-7	PUR	**-**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
P03528A1-10	PUR	**-**	H	S	null	Absent
P0527A1-9	PUR	**-**	L	S	null	Absent
P0537A1-7	PUR	**-**	H	NA	null	Present
P0570A1-2	PUR	**-**	H	I	null	Present
P07469A1-28	PUR	**-**	L	I	null	Absent
P9346A1-2	PUR	**-**	L	S	null	Absent
P99751RA1-6	PUR	**-**	L	S	null	Absent
P99840C4-8	PUR	**-**	L	S	null	Absent
Panola	Agripro	**2005**	L	S	null	Absent
Pat	AR	**2001**	L	S	null	Absent
Patterson	IN	**1995**	L	S	null	Absent
Patton		**1999**	NA	NA	NA	Absent
Pembroke		**-**	H	null	null	Present
Pennoll	PA	**1951**	L	S	null	Absent
PI 94761(*T. timopheevii*)			H	null	null	Present
Pike	MO	**1980**	L	S	null	Absent
Pioneer 2510	IN	**1991**	NA	NA	NA	Absent
Pioneer 2548	IN	**1988**	L	S	null	Absent
Pioneer 2550	IN	**1981**	L	S	null	Absent
Pioneer 2555	IN	**1986**	L	S	null	Absent
Pioneer 2568	IN	**1995**	L	S	null	Absent
Pioneer 2580	IN	**1993**	L	S	null	Absent
Pioneer 25R18	IN	**1999**	L	I	null	Absent
Pioneer 25R26	IN	**1996**	NA	NA	NA	Absent
Pioneer 25R37	IN	**2000**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Pioneer 25R47	IN	**2002**	L	S	null	Absent
Pioneer 25R57	IN	**1996**	L	S	null	Absent
Pioneer 25W60	IN	**1998**	L	S	null	Absent
Pioneer 2643	IN	**1994**	H	null	null	Present
Pioneer 2684	IN	**1993**	H	null	null	Present
Pioneer 26R15	IN	**-**	L	S	null	Absent
Pioneer 26R24	IN	**1999**	L	S	null	Absent
Pioneer 26R31	IN	**2004**	H	null	null	Present
Pioneer 26R46	IN	**1998**	L	S	null	Absent
Pioneer 26R61	IN	**1998**	L	S	null	Absent
Pioneer S-76	IN	**1976**	L	S	null	Absent
Pioneer S-78	IN	**1978**	L	S	null	Absent
Pontiac	IN	**1993**	L	S	NA	Absent
Potomac	VA	**1975**	L	S	null	Absent
Progold		**1993**	H	I	null	Present
Purplestraw	AU	**1822**	L	S	NA	Absent
Red May	US	**1929**	L	S	null	Absent
Redcoat	IN	**1960**	L	S	null	Absent
Redhart Coker	SC	**1929**	L	S	null	Absent
Roane	VA	**1999**	L	S	null	Absent
Roy	NC	**1979**	L	S	null	Absent
Royal	IL	**1947**	L	S	null	Absent
Rudy	OH	**1871**	L	S	null	Absent
Ruler	OH	**1975**	L	S	null	Absent
Rupert Giant	NY	**1917**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Saluda	VA	**1983**	L	S	null	Absent
Saranac	NY	**2008**	NA	NA	NA	Absent
Sawyer	CO	**1991**	L	S	null	Absent
Scotty	IL	**1982**	H	I	null	Present
Seneca	NY	**1950**	L	S	null	Absent
Severn	MD	**1981**	H	I	null	Present
Shiloh	IN	**1996**	L	S	null	Absent
Shirley	VA	**2008**	L	S	null	Absent
Sisson	VA	**2000**	H	I	null	Present
SS 520	GA	**2001**	L	S	null	Absent
SS 5205	GA	**2009**	H	I	null	Present
SS 560	GA	**2002**	L	S	null	Absent
SS8641	GA	**2008**	L	S	null	Absent
Stacey		**1980**	L	S	null	Absent
Steinwedel	AU	**1890**	L	I	NA	Absent
Stoddard	MO	**1973**	L	S	null	Absent
Sullivan	IN	**1977**	H	I	null	Present
Tecumseh	MI	**1973**	H	I	null	Present
Timstein	MN	**1939**	L	I	NA	Absent
Timvera	AU	**1956**	H	null	NA	Present
Titan	OH	**1978**	L	S	null	Absent
Tribute	VA	**2002**	L	S	null	Absent
Truman	MO	**2003**	L	S	null	Absent
Trumbull	OH	**1916**	L	S	null	Absent
Twain	KS	**1987**	L	S	null	Absent

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Tyler	VA	**1980**	L	S	null	Absent
USG 3120		**-**	L	S	I	Absent
USG 3209		**1999**	H	null	null	Present
USG 3295		**-**	H	null	null	Present
USG 3555	VA	**-**	H	null	null	Present
USG 3592		**2004**	L	S	null	Absent
VA 259	VA	**-**	H	null	null	Present
VA 90	VA	**-**	L	S	null	Absent
VA 96W-247	VA	**2003**	NA	NA	NA	Present
VA00W-38	VA	**-**	L	S	null	Absent
VA01W-21	VA	**-**	null	null	null	Absent
VA01W-476	VA	**-**	H	S	null	Absent
VA01W713	VA	**-**	L	S	null	Absent
VA03W-211	VA	**-**	H	null	null	Present
VA03W-235	VA	**-**	L	S	null	Absent
VA05W-139	VA	**-**	L	S	null	Absent
VA05W-151	VA	**-**	L	S	null	Absent
Vermillion (Awnless)	IN	**1955**	L	S	null	Absent
Vermont Winter Reeds		**1894**	L	S	null	Absent
Vigo	IN	**1946**	L	S	null	Absent
Wakefield	VA	**1990**	L	S	null	Absent
Wakeland	NC	**1959**	L	S	null	Absent
Warwick		**2003**	L	S	null	Absent
Wheeler	VA	**1980**	H	I	null	Present

Table 1 Continued

Line name	Location	Registration	*TaSus2-2B*	*TaPpd BJ001*	*TaPpdBJ003*	*Sr36*
Wilson		**2002**	L	S	null	Absent
Wisdom		**2003**	L	S	null	Absent
Wonder		**2002**	L	S	null	Absent
Yorkstar	NY	**1968**	L	S	null	Absent
Yorkwin	NY	**1935**	L	S	null	Absent

Table 2. Mean Polymorphism Information Content (PIC) values of 579 markers on chromosome 2B. PIC values were calculated using subsets of cultivars by date of release and the presence/absence of *Sr36*. Mean PIC values of all 579 markers by subset were calculated, as well as the mean PIC values of the markers in smaller subsets of the chromosome length.

Region of Chromosome 2B	Number of markers	Marker Density (cM/ Marker)	All cultivars	Cultivars released before 1950	Cultivars released after 1950	All cultivars without *Sr36*	All cultivars with *Sr36*
Distal 2BS non-translocation (0 cM to 43.86 cM)	25	1.75	0.40	0.31	0.40	0.40	0.36
2BS unknown (44.81 cM to 102.25 cM)	53	1.08	0.32	0.22	0.33	0.29	0.30
49 translocation specific markers	49	4.06	0.41	0.13	0.43	0.14	0.00
All markers in region of the translocation (104.63 cM to 303.68 cM)	436	0.46	0.37	0.29	0.37	0.32	0.00
Distal 2BL non-translocation (304.01 cM to 395.92 cM)	62	1.48	0.35	0.32	0.35	0.36	0.21
Average PIC whole chromosome (0 cM to 395.92 cM)	579	0.69	0.37	0.28	0.37	0.32	0.07

Table 3. Frequencies of alleles for markers on chromosome 2B in eastern U.S. winter wheat cultivars. Allele frequencies are listed in groupings by release date and/or presence of *Sr36*. Frequencies do not equal 1.00 due to heterozygotes and missing data.

Trait	Marker	Allele	All cultivars	*Sr36*+ cultivars	*Sr36*- cultivars	Pre-1950 cultivars	Post-1950 cultivars	Post-1950, *Sr36*- cultivars
Sucrose Synthase2	*TaSus2-2B*	High TGW	0.26	0.99	0.03	0.00	0.29	0.03
		Low TGW	0.69	0.00	0.92	0.94	0.67	0.92
Photoperiod response (*Ppd-B1*)	TaPpdBJ001, Chinese Spring (CS) allele	Insensitive	0.15	0.49	0.04	0.00	0.17	0.05
		Sensitive	0.69	0.01	0.90	0.94	0.66	0.89
		Null	0.12	0.44	0.01	0.00	0.13	0.02
Photoperiod response (*Ppd-B1*)	TaPpdBJ003, Sonora64 intercopy	Insensitive	0.05	0.00	0.07	0.03	0.06	0.08
		Null (Sensitive)	0.91	0.99	0.88	0.89	0.91	0.88

Figures

Figure 1. A color-coded image of chromosome 2B alleles' parentage in eastern U.S. winter wheat germplasm. The x-axis represents 367 cultivars, sorted left to right from the presence to the absence of *Sr36*. The y-axis represents 576 SNP markers in order by genetic position. The y-axis is color-coded as follows: black indicates non-translocation markers, gray indicates markers possibly on the translocation, the white region is markers on translocation, and the orange indicates translocation-specific markers. Each color in the body of the figure is coded according to the presence of alleles in common with founding and modern cultivars. The uniform section on the left represents the monomorphic markers in 90 cultivars with the translocation. The allele colors are: Mediterranean (dark green), Purplestraw (purple), Fultz (golden yellow), Genesee Giant (red), Harvest Queen (light yellow), Grandprize (light green), NC-Neuse (blue), and AGS 2000 (gray).

APPENDICES

Appendix A. Polymorphism Information Content (PIC) values of 581 markers on chromosome 2B. PIC values were calculated using subsets of cultivars by date of release and the presence of *Sr36*. The mean PIC value of all 581 markers by cultivar subset was calculated. Index values highlighted in orange signify markers with an allele segregating directly with the introgression.

Index	Marker	Position (cM)	All cultivars	Only cultivars with *Sr36*	Only cultivars lacking *Sr36*	Only cultivars released before 1950	Only cultivars released after 1950
6262	wsnp_JD_rep_c49438_33652645	1.51	0.630	0.588	0.633	0.617	0.628
1413	wsnp_Ex_c10961_17803258	2.32	0.494	0.479	0.479	0.208	0.499
7633	wsnp_Ra_c1501_2991585	2.82	0.497	0.474	0.486	0.251	0.500
6263	wsnp_JD_rep_c49438_33652663	3.14	0.500	0.500	0.500	0.500	0.500
4808	wsnp_Ex_c851_1654297	7.15	0.401	0.296	0.432	0.467	0.390
8128	wsnp_Ra_rep_c106727_90434958	7.25	0.473	0.414	0.485	0.408	0.454
4723	wsnp_Ex_c7776_13247365	7.25	0.453	0.444	0.455	0.202	0.467
7656	wsnp_Ra_c1660_3275687	7.25	0.481	0.447	0.488	0.408	0.466
2482	wsnp_Ex_c1996_3754394	7.25	0.481	0.447	0.488	0.408	0.466
2973	wsnp_Ex_c25445_34710489	8.36	0.435	0.346	0.456	0.500	0.419
6138	wsnp_JD_c640_960796	8.47	0.453	0.444	0.456	0.202	0.467
3005	wsnp_Ex_c259_497455	13.34	0.334	0.325	0.336	0.056	0.355
2988	wsnp_Ex_c25688_34949297	21.47	0.425	0.397	0.433	0.298	0.435
7545	wsnp_Ra_c10712_17572884	21.47	0.425	0.431	0.423	0.157	0.442
1799	wsnp_Ex_c13686_21480826	21.47	0.216	0.231	0.211	0.157	0.222
7370	wsnp_Ku_c9883_16462146	21.98	0.302	0.215	0.327	0.157	0.315
6219	wsnp_JD_c930_1368255	22.99	0.045	0.000	0.059	0.108	0.037
2846	wsnp_Ex_c2388_4476302	28.32	0.499	0.500	0.498	0.498	0.499
7936	wsnp_Ra_c4321_7860456	28.32	0.499	0.500	0.498	0.498	0.499

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
5264	wsnp_Ex_rep_c66551_64836327	28.32	0.033	0.022	0.036	0.000	0.036
749	wsnp_CAP11_c3271_1608092	29.81	0.362	0.488	0.291	0.245	0.372
2407	wsnp_Ex_c19371_28311667	29.81	0.439	0.320	0.464	0.415	0.441
6767	wsnp_Ku_c2486_4751695	33.78	0.113	0.064	0.128	0.202	0.103
5137	wsnp_Ex_rep_c105401_89840110	34.79	0.411	0.263	0.444	0.415	0.411
8124	wsnp_Ra_rep_c106119_89961852	43.86	0.478	0.358	0.495	0.382	0.484
8152	wsnp_Ra_rep_c117300_96881829	44.81	0.499	0.470	0.499	0.389	0.500
2110	wsnp_Ex_c1629_3103725	50.75	0.074	0.180	0.036	0.000	0.081
2111	wsnp_Ex_c1629_3103807	50.75	0.074	0.180	0.036	0.000	0.081
2112	wsnp_Ex_c1629_3104356	50.75	0.064	0.143	0.036	0.000	0.070
5554	wsnp_Ex_rep_c68623_67474885	50.75	0.074	0.180	0.036	0.000	0.081
5555	wsnp_Ex_rep_c68623_67474935	50.75	0.069	0.164	0.036	0.000	0.076
7106	wsnp_Ku_c48_103915	50.75	0.074	0.180	0.036	0.000	0.081
2088	wsnp_Ex_c1602_3055066	50.75	0.074	0.180	0.036	0.000	0.081
7697	wsnp_Ra_c19083_28215239	55.26	0.486	0.498	0.470	0.431	0.489
888	wsnp_CAP11_rep_c8489_3664985	56.9	0.316	0.105	0.368	0.108	0.334
889	wsnp_CAP11_rep_c8489_3665230	56.9	0.472	0.500	0.450	0.157	0.485
6048	wsnp_JD_c42879_30043973	56.9	0.472	0.500	0.450	0.157	0.485
1930	wsnp_Ex_c14711_22788586	64.09	0.469	0.499	0.443	0.245	0.480
3838	wsnp_Ex_c4272_7708423	64.09	0.027	0.000	0.035	0.000	0.030
1929	wsnp_Ex_c14711_22788263	64.09	0.469	0.499	0.443	0.245	0.480
2116	wsnp_Ex_c163_320858	64.54	0.027	0.000	0.035	0.000	0.030
2117	wsnp_Ex_c163_321026	64.54	0.500	0.499	0.500	0.500	0.500

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
2115	wsnp_Ex_c163_320267	64.54	0.027	0.000	0.035	0.000	0.030
5737	wsnp_Ex_rep_c72527_70882805	66.93	0.068	0.085	0.063	0.056	0.070
1931	wsnp_Ex_c14711_22789762	66.93	0.068	0.085	0.063	0.056	0.070
5708	wsnp_Ex_rep_c70756_69644826	66.93	0.476	0.499	0.454	0.245	0.486
7799	wsnp_Ra_c265_560747	68.29	0.496	0.491	0.488	0.496	0.495
5721	wsnp_Ex_rep_c71023_69867676	68.79	0.495	0.492	0.485	0.500	0.494
2440	wsnp_Ex_c1962_3696265	69.12	0.496	0.492	0.487	0.496	0.494
7120	wsnp_Ku_c4962_8872507	69.12	0.495	0.492	0.485	0.500	0.494
2443	wsnp_Ex_c1962_3697716	69.12	0.496	0.492	0.487	0.496	0.494
4285	wsnp_Ex_c56027_58308093	69.85	0.439	0.498	0.403	0.496	0.429
4284	wsnp_Ex_c56027_58306755	69.85	0.404	0.498	0.344	0.490	0.390
6085	wsnp_JD_c5064_6183978	70	0.475	0.500	0.454	0.245	0.485
6184	wsnp_JD_c8085_9129899	70.13	0.335	0.496	0.231	0.056	0.356
1360	wsnp_Ex_c10596_17293363	77.23	0.475	0.044	0.318	0.202	0.486
1359	wsnp_Ex_c10596_17293192	77.23	0.399	0.500	0.318	0.202	0.414
413	wsnp_BF146221B_Ta_2_2	77.23	0.000	0.000	0.000	0.000	0.000
7916	wsnp_Ra_c407_862316	77.47	0.423	0.498	0.374	0.056	0.444
6474	wsnp_Ku_c12721_20478606	77.77	0.475	0.044	0.318	0.202	0.486
2391	wsnp_Ex_c19260_28187434	77.77	0.436	0.532	0.333	0.202	0.455
2572	wsnp_Ex_c21092_30220702	81.83	0.466	0.022	0.500	0.408	0.444
2571	wsnp_Ex_c21092_30220342	81.83	0.499	0.498	0.500	0.408	0.496
546	wsnp_BG275030A_Td_2_1	81.83	0.000	0.000	0.000	0.000	0.000
5697	wsnp_Ex_rep_c70571_69488416	88.04	0.493	0.000	0.485	0.467	0.488
4652	wsnp_Ex_c7285_12506938	89.06	0.376	0.497	0.300	0.320	0.382

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
4421	wsnp_Ex_c6099_10674508	89.56	0.299	0.497	0.170	0.284	0.301
4420	wsnp_Ex_c6099_10674406	89.56	0.299	0.497	0.170	0.284	0.301
4554	wsnp_Ex_c66052_64232430	89.78	0.132	0.000	0.170	0.284	0.113
7069	wsnp_Ku_c44441_51721669	92.03	0.136	0.000	0.176	0.056	0.144
6943	wsnp_Ku_c34759_44069854	92.03	0.500	0.497	0.498	0.438	0.498
6740	wsnp_Ku_c23305_33210841	92.03	0.303	0.497	0.176	0.056	0.323
1407	wsnp_Ex_c10941_17776989	92.03	0.255	0.497	0.049	0.000	0.277
6026	wsnp_JD_c3732_4781170	92.03	0.496	0.497	0.491	0.498	0.495
6739	wsnp_Ku_c23305_33210628	92.03	0.303	0.497	0.176	0.056	0.323
6893	wsnp_Ku_c31_62657	92.03	0.467	0.000	0.500	0.438	0.469
8381	wsnp_RFL_Contig2744_2471775	99.43	0.444	0.000	0.210	0.202	0.459
8599	wsnp_RFL_Contig4483_5312236	102.25	0.499	0.000	0.468	0.108	0.488
5392	wsnp_Ex_rep_c67391_65971023	104.63	0.387	0.000	0.454	0.480	0.350
6364	wsnp_JG_c609_370792	105.74	0.478	0.000	0.318	0.245	0.488
2624	wsnp_Ex_c2153_4043746	106	0.132	0.000	0.170	0.056	0.139
5147	wsnp_Ex_rep_c106004_90240692	106	0.499	0.000	0.467	0.496	0.499
3126	wsnp_Ex_c27867_37030045	106	0.499	0.000	0.467	0.496	0.499
1763	wsnp_Ex_c13351_21042379	106	0.499	0.000	0.467	0.496	0.499
6509	wsnp_Ku_c13905_22034406	106	0.404	0.000	0.467	0.496	0.378
7661	wsnp_Ra_c16822_25566950	106	0.499	0.000	0.467	0.496	0.499
3564	wsnp_Ex_c3614_6602465	109.62	0.418	0.000	0.118	0.056	0.440
2312	wsnp_Ex_c18418_27251563	110.75	0.127	0.000	0.164	0.056	0.134
4456	wsnp_Ex_c62404_62055681	110.75	0.117	0.000	0.152	0.056	0.124
762	wsnp_CAP11_c3947_1866837	110.75	0.428	0.000	0.158	0.056	0.449

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
5753	wsnp_Ex_rep_c81490_76243612	110.75	0.127	0.000	0.164	0.056	0.134
763	wsnp_CAP11_c3947_1867089	110.75	0.122	0.000	0.158	0.056	0.129
2887	wsnp_Ex_c2430_4546479	110.75	0.428	0.000	0.158	0.056	0.449
4673	wsnp_Ex_c741_1456698	110.75	0.499	0.000	0.425	0.490	0.499
608	wsnp_BM140364B_Ta_2_3	110.75	0.127	0.000	0.164	0.056	0.134
607	wsnp_BM140364B_Ta_2_1	110.75	0.127	0.000	0.164	0.056	0.134
295	wsnp_BE497494B_Ta_2_1	110.75	0.428	0.000	0.158	0.056	0.449
5818	wsnp_JD_c12687_12877994	110.75	0.499	0.000	0.425	0.490	0.499
2556	wsnp_Ex_c20786_29874875	111.26	0.117	0.000	0.152	0.056	0.124
2557	wsnp_Ex_c20786_29875033	111.26	0.122	0.000	0.158	0.056	0.129
4655	wsnp_Ex_c7324_12561727	111.26	0.122	0.000	0.158	0.056	0.129
5560	wsnp_Ex_rep_c68704_67559626	111.26	0.117	0.000	0.152	0.056	0.124
7030	wsnp_Ku_c4042_7375890	111.26	0.065	0.000	0.086	0.056	0.066
1665	wsnp_Ex_c12671_20140295	112.95	0.117	0.000	0.152	0.056	0.124
1664	wsnp_Ex_c12671_20140014	112.95	0.428	0.000	0.158	0.056	0.449
4531	wsnp_Ex_c6537_11338763	117.14	0.117	0.000	0.152	0.056	0.124
7029	wsnp_Ku_c4042_7375053	117.65	0.122	0.000	0.158	0.056	0.129
3329	wsnp_Ex_c31274_40086873	118.15	0.127	0.000	0.164	0.056	0.134
8182	wsnp_Ra_rep_c71728_69843225	118.15	0.127	0.000	0.164	0.056	0.134
8221	wsnp_RFL_Contig1074_103556	119.67	0.132	0.000	0.170	0.056	0.139
897	wsnp_CAP11_rep_c8700_3756682	120.68	0.136	0.000	0.176	0.056	0.144
3127	wsnp_Ex_c27867_37030229	127.29	0.303	0.000	0.370	0.467	0.277
4642	wsnp_Ex_c7246_12443506	131.87	0.231	0.000	0.291	0.202	0.234
3278	wsnp_Ex_c30447_39360584	131.87	0.011	0.000	0.014	0.056	0.006

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
4720	wsnp_Ex_c7738_13195349	132.37	0.194	0.000	0.247	0.157	0.198
7076	wsnp_Ku_c4507_8157580	134.94	0.446	0.000	0.210	0.056	0.465
1114	wsnp_CAP7_rep_c12606_5316797	136.95	0.426	0.000	0.152	0.056	0.447
429	wsnp_BF202681B_Ta_2_2	136.95	0.446	0.000	0.210	0.056	0.465
2671	wsnp_Ex_c22010_31185837	136.95	0.496	0.000	0.405	0.202	0.500
6427	wsnp_Ku_c11665_18999583	137.45	0.498	0.000	0.471	0.496	0.497
5377	wsnp_Ex_rep_c67257_65786614	137.45	0.485	0.000	0.361	0.202	0.494
328	wsnp_BE499478B_Ta_2_1	137.45	0.494	0.000	0.388	0.202	0.499
1010	wsnp_CAP12_rep_c4678_2134259	154.8	0.011	0.000	0.014	0.000	0.012
2670	wsnp_Ex_c22010_31185638	155.86	0.551	0.000	0.433	0.207	0.564
8555	wsnp_RFL_Contig4076_4580964	159.97	0.471	0.043	0.301	0.057	0.486
6010	wsnp_JD_c33633_25567951	160.48	0.370	0.000	0.000	0.000	0.395
7622	wsnp_Ra_c14034_22046454	160.48	0.370	0.000	0.000	0.000	0.395
8056	wsnp_Ra_c7093_12292610	160.48	0.370	0.000	0.000	0.000	0.395
2138	wsnp_Ex_c16496_25002840	160.48	0.370	0.000	0.000	0.000	0.395
8068	wsnp_Ra_c7699_13203536	160.98	0.370	0.000	0.000	0.000	0.395
10	wsnp_BE399688B_Ta_2_1	161.48	0.428	0.000	0.484	0.353	0.387
575	wsnp_BG606636A_Ta_1_1	161.48	0.370	0.000	0.000	0.000	0.395
6188	wsnp_JD_c8219_9245110	161.48	0.371	0.000	0.000	0.000	0.396
7059	wsnp_Ku_c4373_7947422	161.48	0.370	0.000	0.000	0.000	0.395
1914	wsnp_Ex_c14595_22634031	161.48	0.053	0.000	0.070	0.000	0.059
8367	wsnp_RFL_Contig2666_2354537	161.48	0.430	0.000	0.164	0.056	0.451
1508	wsnp_Ex_c11506_18551574	161.48	0.370	0.000	0.000	0.000	0.395
2525	wsnp_Ex_c20323_29388970	161.48	0.370	0.000	0.000	0.000	0.395

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
4323	wsnp_Ex_c57_116914	161.48	0.478	0.000	0.318	0.202	0.489
4807	wsnp_Ex_c8505_14301862	161.48	0.370	0.000	0.000	0.000	0.395
5155	wsnp_Ex_rep_c107482_91099005	161.48	0.370	0.000	0.000	0.000	0.395
5829	wsnp_JD_c13299_13330728	161.48	0.370	0.000	0.000	0.000	0.395
6423	wsnp_Ku_c11488_18750063	161.48	0.370	0.000	0.000	0.000	0.395
6446	wsnp_Ku_c11972_19450608	161.48	0.370	0.000	0.000	0.000	0.395
7163	wsnp_Ku_c5465_9696838	161.48	0.370	0.000	0.000	0.000	0.395
3080	wsnp_Ex_c269_517710	161.48	0.430	0.000	0.164	0.056	0.451
3924	wsnp_Ex_c45094_50985067	161.48	0.494	0.000	0.484	0.353	0.498
3081	wsnp_Ex_c269_518324	161.48	0.430	0.000	0.164	0.056	0.451
561	wsnp_BG314205D_Ta_2_3	161.48	0.370	0.000	0.000	0.000	0.395
3678	wsnp_Ex_c3937_7140823	161.48	0.370	0.000	0.000	0.000	0.395
4894	wsnp_Ex_c9248_15372536	161.48	0.495	0.000	0.482	0.408	0.498
8085	wsnp_Ra_c8567_14485811	161.48	0.370	0.000	0.000	0.000	0.395
6462	wsnp_Ku_c12517_20191465	161.48	0.488	0.000	0.492	0.284	0.472
6430	wsnp_Ku_c1176_2350548	161.48	0.372	0.022	0.007	0.000	0.397
652	wsnp_CAP11_c114_140053	161.48	0.378	0.000	0.014	0.056	0.400
3277	wsnp_Ex_c3044_5620102	163.67	0.371	0.000	0.440	0.467	0.357
6135	wsnp_JD_c6350_7516597	163.67	0.488	0.000	0.492	0.408	0.477
8344	wsnp_RFL_Contig2506_2098552	163.67	0.016	0.000	0.021	0.000	0.018
3428	wsnp_Ex_c33246_41764093	163.67	0.439	0.000	0.491	0.408	0.442
2673	wsnp_Ex_c22018_31192778	163.67	0.494	0.000	0.483	0.490	0.495
6547	wsnp_Ku_c15057_23554067	163.67	0.494	0.000	0.483	0.490	0.495
4102	wsnp_Ex_c51461_55394646	163.67	0.431	0.000	0.486	0.284	0.442

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
1087	wsnp_CAP7_c402_217331	163.67	0.439	0.000	0.491	0.408	0.442
5948	wsnp_JD_c2367_3220232	163.67	0.500	0.000	0.451	0.467	0.499
6075	wsnp_JD_c4699_5834958	163.67	0.488	0.000	0.492	0.382	0.476
6308	wsnp_JD_rep_c64505_41132927	163.67	0.466	0.000	0.500	0.480	0.464
5811	wsnp_JD_c1236_1789566	163.67	0.487	0.000	0.494	0.284	0.469
6136	wsnp_JD_c6350_7517130	163.67	0.439	0.000	0.491	0.408	0.442
2674	wsnp_Ex_c22018_31193171	163.67	0.365	0.000	0.434	0.467	0.350
6554	wsnp_Ku_c15336_23908130	163.67	0.466	0.000	0.500	0.480	0.464
5038	wsnp_Ex_rep_c101906_87187119	163.67	0.439	0.000	0.491	0.408	0.442
50	wsnp_BE404601B_Ta_2_1	163.67	0.494	0.000	0.483	0.490	0.495
905	wsnp_CAP11_rep_c9018_3888047	163.67	0.248	0.000	0.310	0.353	0.235
7263	wsnp_Ku_c7096_12264232	163.67	0.483	0.000	0.497	0.382	0.467
4472	wsnp_Ex_c62844_62315607	163.67	0.365	0.000	0.434	0.467	0.350
4915	wsnp_Ex_c944_1810245	163.67	0.365	0.000	0.434	0.467	0.350
5464	wsnp_Ex_rep_c67865_66570323	164.23	0.487	0.000	0.493	0.408	0.476
6830	wsnp_Ku_c27759_37702654	165.37	0.437	0.000	0.489	0.408	0.440
3621	wsnp_Ex_c3764_6853627	166.53	0.500	0.000	0.438	0.382	0.500
4984	wsnp_Ex_c9935_16358536	167.08	0.371	0.000	0.440	0.382	0.370
6664	wsnp_Ku_c18923_28319203	167.08	0.357	0.000	0.426	0.382	0.354
4983	wsnp_Ex_c9935_16358436	167.08	0.500	0.000	0.440	0.382	0.499
2977	wsnp_Ex_c2557_4761174	167.08	0.371	0.000	0.440	0.382	0.370
5149	wsnp_Ex_rep_c106085_90293854	167.08	0.621	0.000	0.440	0.382	0.633
3213	wsnp_Ex_c29108_38173309	167.63	0.387	0.000	0.454	0.408	0.385
6818	wsnp_Ku_c2725_5163091	167.63	0.376	0.000	0.444	0.408	0.372

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
1154	wsnp_CAP8_c1591_913693	168.14	0.370	0.000	0.000	0.000	0.395
3154	wsnp_Ex_c28252_37391595	168.64	0.370	0.000	0.000	0.000	0.395
34	wsnp_BE403597B_Ta_1_1	170.15	0.374	0.000	0.007	0.000	0.400
4135	wsnp_Ex_c5239_9272511	170.65	0.497	0.000	0.411	0.451	0.499
201	wsnp_be471201A_Ta_1_3	171.15	0.005	0.000	0.007	0.056	0.000
5850	wsnp_JD_c1472_2090800	173.66	0.370	0.000	0.000	0.000	0.395
4303	wsnp_Ex_c56417_58543388	174.17	0.362	0.000	0.000	0.000	0.387
5506	wsnp_Ex_rep_c68173_66946365	174.67	0.399	0.000	0.076	0.000	0.423
1179	wsnp_CAP8_c303_286918	177.18	0.615	0.000	0.429	0.245	0.633
4388	wsnp_Ex_c5943_10422207	177.68	0.389	0.000	0.049	0.000	0.414
1938	wsnp_Ex_c14771_22883575	183.39	0.499	0.000	0.429	0.245	0.499
2885	wsnp_Ex_c24273_33514325	183.39	0.500	0.024	0.451	0.408	0.499
3622	wsnp_Ex_c3769_6861195	183.39	0.500	0.000	0.444	0.408	0.499
6819	wsnp_Ku_c2725_5163093	183.39	0.000	0.000	0.000	0.000	0.000
6781	wsnp_Ku_c2562_4879681	183.39	0.374	0.000	0.442	0.408	0.370
7951	wsnp_Ra_c46023_52004149	183.39	0.500	0.000	0.442	0.408	0.499
6476	wsnp_Ku_c12900_20727771	183.39	0.360	0.000	0.429	0.245	0.370
1059	wsnp_CAP7_c2233_1085003	183.39	0.360	0.000	0.429	0.245	0.370
439	wsnp_BF291736B_Ta_1_1	183.39	0.360	0.000	0.429	0.245	0.370
3656	wsnp_Ex_c38739_46195930	183.39	0.499	0.000	0.429	0.245	0.499
2151	wsnp_Ex_c16582_25102216	183.92	0.389	0.000	0.049	0.000	0.414
7821	wsnp_Ra_c2842_5399988	183.97	0.142	0.000	0.184	0.056	0.151
1188	wsnp_CAP8_c4328_2115116	183.97	0.232	0.000	0.292	0.408	0.208
5436	wsnp_Ex_rep_c67671_66332423	183.97	0.451	0.000	0.496	0.480	0.432

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
4100	wsnp_Ex_c51352_55323092	183.97	0.482	0.000	0.497	0.480	0.483
771	wsnp_CAP11_c5240_2436441	183.97	0.235	0.000	0.296	0.408	0.212
874	wsnp_CAP11_rep_c5367_2492424	183.97	0.236	0.000	0.296	0.408	0.212
1102	wsnp_CAP7_c7341_3280398	183.97	0.235	0.000	0.296	0.408	0.212
1128	wsnp_CAP7_rep_c7219_3228859	183.97	0.232	0.000	0.292	0.408	0.208
1129	wsnp_CAP7_rep_c7347_3283296	183.97	0.235	0.000	0.296	0.408	0.212
6209	wsnp_JD_c892_1313553	183.97	0.454	0.000	0.497	0.480	0.437
5724	wsnp_Ex_rep_c71116_69944350	183.97	0.438	0.000	0.490	0.480	0.432
4605	wsnp_Ex_c7003_12065642	183.97	0.453	0.000	0.497	0.467	0.452
1237	wsnp_CAP8_rep_c5599_2654989	183.97	0.235	0.000	0.296	0.408	0.212
170	wsnp_BE445278B_Ta_2_3	183.97	0.160	0.000	0.205	0.056	0.170
2344	wsnp_Ex_c1873_3531947	183.97	0.032	0.000	0.042	0.108	0.024
4604	wsnp_Ex_c7003_12065567	183.97	0.384	0.000	0.451	0.451	0.375
4606	wsnp_Ex_c7003_12065828	183.97	0.384	0.000	0.451	0.451	0.375
535	wsnp_BG263521B_Ta_2_1	183.97	0.424	0.000	0.482	0.451	0.421
2766	wsnp_Ex_c22946_32163010	183.97	0.424	0.000	0.482	0.451	0.421
776	wsnp_CAP11_c5474_2542512	183.97	0.235	0.000	0.296	0.408	0.212
7520	wsnp_Ku_rep_c73313_72887199	183.97	0.495	0.000	0.482	0.451	0.490
1145	wsnp_CAP8_c1240_753371	183.97	0.016	0.000	0.021	0.000	0.018
169	wsnp_BE445278B_Ta_2_1	183.97	0.205	0.000	0.205	0.056	0.224
8517	wsnp_RFL_Contig3802_4108582	184.4	0.468	0.000	0.500	0.490	0.456
1981	wsnp_Ex_c15089_23270614	184.61	0.461	0.000	0.499	0.480	0.445
2714	wsnp_Ex_c22423_31615798	184.61	0.461	0.000	0.499	0.480	0.445
3860	wsnp_Ex_c4328_7800187	184.61	0.461	0.000	0.499	0.480	0.445

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
1980	wsnp_Ex_c15089_23270403	184.61	0.461	0.000	0.499	0.480	0.445
3862	wsnp_Ex_c4328_7801724	184.61	0.461	0.000	0.499	0.480	0.445
3861	wsnp_Ex_c4328_7801438	184.61	0.461	0.000	0.499	0.480	0.445
6918	wsnp_Ku_c33287_42805533	184.61	0.461	0.000	0.499	0.480	0.445
5017	wsnp_Ex_rep_c101477_86838533	187.49	0.499	0.000	0.465	0.408	0.495
2543	wsnp_Ex_c20529_29608872	187.49	0.492	0.000	0.488	0.490	0.488
6778	wsnp_Ku_c2561_4878036	189.9	0.449	0.105	0.247	0.000	0.469
2025	wsnp_Ex_c1541_2943791	193.46	0.498	0.000	0.227	0.108	0.522
2024	wsnp_Ex_c1541_2943368	193.96	0.221	0.000	0.221	0.108	0.236
3817	wsnp_Ex_c4218_7618252	194.46	0.490	0.000	0.210	0.108	0.514
5246	wsnp_Ex_rep_c66482_64735570	194.96	0.032	0.000	0.042	0.000	0.035
4134	wsnp_Ex_c5238_9271937	194.96	0.492	0.000	0.488	0.490	0.488
3453	wsnp_Ex_c3386_6217891	194.96	0.489	0.000	0.489	0.490	0.489
1661	wsnp_Ex_c12634_20096724	195.47	0.499	0.000	0.467	0.408	0.495
6970	wsnp_Ku_c37857_46627257	195.97	0.449	0.022	0.226	0.157	0.465
2050	wsnp_Ex_c1568_2993540	195.97	0.492	0.000	0.488	0.490	0.488
5247	wsnp_Ex_rep_c66482_64735785	195.97	0.488	0.000	0.493	0.490	0.483
3554	wsnp_Ex_c36002_44045355	195.97	0.449	0.000	0.221	0.108	0.466
5261	wsnp_Ex_rep_c66545_64828871	195.97	0.492	0.000	0.488	0.490	0.488
5248	wsnp_Ex_rep_c66482_64736708	195.97	0.488	0.000	0.493	0.490	0.483
433	wsnp_BF202975B_Ta_2_1	196.47	0.492	0.000	0.488	0.490	0.488
4696	wsnp_Ex_c7516_12850225	196.97	0.492	0.000	0.488	0.490	0.488
2625	wsnp_Ex_c21532_30680512	196.97	0.449	0.000	0.221	0.108	0.466
4014	wsnp_Ex_c482_957514	196.97	0.449	0.000	0.221	0.108	0.466

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
7251	wsnp_Ku_c691_1429427	197.48	0.448	0.022	0.221	0.157	0.464
7499	wsnp_Ku_rep_c71678_71421327	197.98	0.492	0.000	0.488	0.490	0.488
3942	wsnp_Ex_c45595_51343969	197.98	0.494	0.000	0.483	0.490	0.491
8112	wsnp_Ra_rep_c103071_87770006	197.98	0.494	0.000	0.483	0.490	0.491
6438	wsnp_Ku_c11850_19271281	198.48	0.490	0.000	0.491	0.480	0.484
4473	wsnp_Ex_c6295_10966854	198.98	0.489	0.000	0.492	0.490	0.484
6639	wsnp_Ku_c1793_3506600	198.98	0.492	0.000	0.488	0.490	0.488
5658	wsnp_Ex_rep_c69928_68889412	199.99	0.442	0.000	0.199	0.108	0.460
7919	wsnp_Ra_c4126_7552133	200.49	0.449	0.000	0.221	0.108	0.466
5653	wsnp_Ex_rep_c69895_68855724	200.99	0.492	0.000	0.488	0.490	0.488
2349	wsnp_Ex_c18750_27627600	200.99	0.494	0.000	0.483	0.490	0.491
2184	wsnp_Ex_c16988_25586811	201.5	0.005	0.000	0.007	0.056	0.000
5654	wsnp_Ex_rep_c69895_68856078	202	0.492	0.000	0.488	0.490	0.488
8454	wsnp_RFL_Contig3304_3372802	202	0.492	0.000	0.488	0.490	0.488
4136	wsnp_Ex_c52405_56014689	202	0.492	0.000	0.488	0.490	0.488
4881	wsnp_Ex_c9133_15199135	202.5	0.449	0.022	0.226	0.157	0.465
4224	wsnp_Ex_c5429_9593668	202.5	0.490	0.000	0.491	0.480	0.484
5254	wsnp_Ex_rep_c66524_64798744	203	0.005	0.000	0.007	0.056	0.000
6216	wsnp_JD_c9251_10121369	203.51	0.499	0.000	0.461	0.408	0.496
7489	wsnp_Ku_rep_c71198_70910111	204.01	0.489	0.000	0.492	0.490	0.484
8227	wsnp_RFL_Contig1150_196572	204.51	0.492	0.000	0.488	0.490	0.488
1309	wsnp_Ex_c10279_16851747	205.01	0.449	0.022	0.226	0.157	0.465
3696	wsnp_Ex_c39862_47046812	205.01	0.492	0.000	0.488	0.490	0.488
7420	wsnp_Ku_rep_c107413_92888678	205.52	0.492	0.000	0.488	0.490	0.488

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
389	wsnp_BE604861B_Ta_2_1	206.02	0.005	0.000	0.007	0.056	0.000
5678	wsnp_Ex_rep_c70228_69172301	206.52	0.490	0.000	0.491	0.480	0.484
6016	wsnp_JD_c352_546108	207.02	0.499	0.000	0.467	0.408	0.495
5926	wsnp_JD_c21530_18862386	207.53	0.492	0.000	0.488	0.490	0.488
1550	wsnp_Ex_c11904_19092123	208.03	0.488	0.000	0.493	0.490	0.483
5723	wsnp_Ex_rep_c71064_69904031	208.53	0.490	0.000	0.490	0.480	0.485
3258	wsnp_Ex_c30037_39004913	208.53	0.449	0.000	0.221	0.108	0.466
671	wsnp_CAP11_c1436_808089	209.03	0.494	0.000	0.483	0.490	0.491
2939	wsnp_Ex_c2497_4666005	209.54	0.449	0.000	0.221	0.108	0.466
2081	wsnp_Ex_c15985_24399118	210.04	0.428	0.022	0.164	0.157	0.445
7215	wsnp_Ku_c6183_10867720	210.54	0.494	0.000	0.483	0.490	0.491
6966	wsnp_Ku_c37269_46153087	211.05	0.499	0.000	0.461	0.408	0.496
3452	wsnp_Ex_c3386_6217645	211.55	0.492	0.000	0.488	0.490	0.488
1935	wsnp_Ex_c14735_22823373	212.05	0.449	0.000	0.221	0.108	0.466
4399	wsnp_Ex_c59991_60628628	212.05	0.490	0.000	0.491	0.480	0.484
4880	wsnp_Ex_c9133_15198714	212.05	0.490	0.000	0.490	0.480	0.485
3153	wsnp_Ex_c28243_37383894	212.55	0.430	0.000	0.164	0.108	0.449
742	wsnp_CAP11_c307_255609	213.06	0.492	0.000	0.488	0.490	0.488
4464	wsnp_Ex_c6248_10896799	213.56	0.490	0.000	0.491	0.480	0.484
2739	wsnp_Ex_c22693_31898036	213.56	0.449	0.022	0.226	0.157	0.465
310	wsnp_be498599B_Ta_1_1	214.06	0.491	0.000	0.489	0.493	0.487
586	wsnp_BG608232B_Ta_2_1	214.56	0.499	0.000	0.467	0.408	0.495
7269	wsnp_Ku_c7188_12418573	215.07	0.449	0.022	0.226	0.157	0.465
3210	wsnp_Ex_c2905_5358140	215.07	0.449	0.000	0.221	0.108	0.466

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
5131	wsnp_Ex_rep_c105160_89664213	216.07	0.488	0.000	0.493	0.490	0.483
5259	wsnp_Ex_rep_c66545_64828225	216.07	0.492	0.000	0.488	0.490	0.488
6723	wsnp_Ku_c22272_32093892	216.57	0.449	0.022	0.226	0.157	0.465
5575	wsnp_Ex_rep_c69016_67915892	216.57	0.490	0.000	0.491	0.480	0.484
6505	wsnp_Ku_c13756_21844262	216.57	0.449	0.000	0.221	0.108	0.466
1392	wsnp_Ex_c10829_17619500	216.57	0.490	0.000	0.491	0.480	0.484
3236	wsnp_Ex_c29434_38471452	217.08	0.500	0.000	0.455	0.408	0.498
7204	wsnp_Ku_c60592_62437239	217.08	0.430	0.000	0.164	0.108	0.449
837	wsnp_CAP11_rep_c4105_1940985	217.08	0.500	0.000	0.459	0.408	0.497
6769	wsnp_Ku_c24932_34902009	217.58	0.173	0.000	0.221	0.108	0.179
2665	wsnp_Ex_c21991_31162645	217.58	0.434	0.000	0.488	0.496	0.423
5659	wsnp_Ex_rep_c69928_68889568	217.58	0.434	0.000	0.488	0.496	0.423
4882	wsnp_Ex_c9133_15199227	217.58	0.173	0.000	0.221	0.108	0.179
7103	wsnp_Ku_c47082_53641298	217.58	0.127	0.000	0.164	0.108	0.129
7252	wsnp_Ku_c691_1429483	217.58	0.173	0.000	0.221	0.108	0.179
1210	wsnp_CAP8_c7453_3441062	217.58	0.073	0.000	0.096	0.000	0.081
6948	wsnp_Ku_c35215_44455846	217.58	0.401	0.000	0.465	0.408	0.401
5091	wsnp_Ex_rep_c103248_88252281	217.58	0.492	0.000	0.488	0.490	0.488
838	wsnp_CAP11_rep_c4105_1941066	217.58	0.132	0.000	0.170	0.108	0.134
2030	wsnp_Ex_c1548_2961101	217.58	0.173	0.000	0.221	0.108	0.179
2760	wsnp_Ex_c22875_32089292	217.58	0.168	0.000	0.215	0.108	0.174
2940	wsnp_Ex_c2497_4666526	217.58	0.173	0.000	0.221	0.108	0.179
3045	wsnp_Ex_c26375_35620271	217.58	0.159	0.000	0.204	0.108	0.164
3618	wsnp_Ex_c37611_45330886	217.58	0.173	0.000	0.221	0.108	0.179

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
8601	wsnp_RFL_Contig454_5360785	217.58	0.370	0.000	0.000	0.000	0.395
1217	wsnp_CAP8_c775_527823	217.58	0.420	0.000	0.479	0.408	0.421
2183	wsnp_Ex_c16970_25563701	217.58	0.434	0.000	0.488	0.490	0.426
2236	wsnp_Ex_c17576_26303707	217.58	0.401	0.000	0.465	0.408	0.401
2464	wsnp_Ex_c19819_28826877	217.58	0.434	0.000	0.488	0.490	0.426
4982	wsnp_Ex_c9932_16354389	217.58	0.426	0.000	0.483	0.490	0.416
5600	wsnp_Ex_rep_c69340_68274022	217.58	0.434	0.000	0.488	0.490	0.426
6240	wsnp_JD_c9810_10594505	217.58	0.401	0.000	0.465	0.408	0.401
6607	wsnp_Ku_c16865_25822301	217.58	0.401	0.000	0.465	0.408	0.401
7146	wsnp_Ku_c52199_57246606	217.58	0.434	0.000	0.488	0.490	0.426
7684	wsnp_Ra_c18396_27453775	217.58	0.401	0.000	0.465	0.408	0.401
31	wsnp_BE403506B_Ta_1_2	217.58	0.492	0.000	0.488	0.490	0.488
3840	wsnp_Ex_c4273_7712252	217.58	0.499	0.000	0.465	0.408	0.495
1056	wsnp_CAP7_c193_107526	217.58	0.173	0.000	0.221	0.108	0.179
7376	wsnp_Ku_rep_c100990_88230610	217.58	0.173	0.000	0.221	0.108	0.179
6265	wsnp_JD_rep_c49813_33962513	217.58	0.422	0.000	0.481	0.484	0.413
7015	wsnp_Ku_c39797_48152615	217.58	0.401	0.000	0.465	0.408	0.401
5983	wsnp_JD_c29301_23358365	217.58	0.401	0.000	0.465	0.408	0.401
1549	wsnp_Ex_c11904_19091967	217.58	0.173	0.000	0.221	0.108	0.179
2544	wsnp_Ex_c20529_29609310	217.58	0.492	0.000	0.488	0.490	0.488
5290	wsnp_Ex_rep_c66700_65028839	217.58	0.396	0.000	0.461	0.408	0.395
4106	wsnp_Ex_c51661_55531646	217.58	0.426	0.000	0.483	0.490	0.416
4107	wsnp_Ex_c51661_55533018	217.58	0.426	0.000	0.483	0.490	0.416
7195	wsnp_Ku_c58640_61287250	217.58	0.401	0.000	0.465	0.408	0.401

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
7524	wsnp_Ku_rep_c83117_78896856	217.58	0.434	0.000	0.488	0.490	0.426
8368	wsnp_RFL_Contig2669_2358747	217.58	0.401	0.000	0.465	0.408	0.401
4189	wsnp_Ex_c5363_9482943	217.58	0.159	0.000	0.204	0.108	0.164
4660	wsnp_Ex_c7347_12594320	217.58	0.173	0.000	0.221	0.108	0.179
5117	wsnp_Ex_rep_c104478_89183627	217.58	0.122	0.000	0.158	0.108	0.124
5168	wsnp_Ex_rep_c108679_91802578	217.58	0.141	0.000	0.182	0.108	0.144
207	wsnp_BE488220B_Ta_1_1	217.58	0.173	0.000	0.221	0.108	0.179
697	wsnp_CAP11_c1956_1045077	217.58	0.122	0.000	0.158	0.108	0.124
3995	wsnp_Ex_c47661_52802548	217.58	0.173	0.000	0.221	0.108	0.179
6215	wsnp_JD_c9251_10121113	217.58	0.127	0.000	0.164	0.108	0.129
438	wsnp_BF291674B_Ta_2_1	217.58	0.073	0.000	0.096	0.000	0.081
772	wsnp_CAP11_c5255_2442548	217.58	0.420	0.000	0.479	0.408	0.421
4965	wsnp_Ex_c9805_16183499	217.58	0.434	0.000	0.488	0.490	0.426
5262	wsnp_Ex_rep_c66545_64829026	217.58	0.434	0.000	0.488	0.490	0.426
5610	wsnp_Ex_rep_c69465_68405569	217.58	0.434	0.000	0.488	0.490	0.426
5253	wsnp_Ex_rep_c66524_64798384	217.58	0.373	0.000	0.007	0.056	0.395
326	wsnp_be499362B_Td_2_1	217.58	0.428	0.000	0.158	0.108	0.447
1216	wsnp_CAP8_c775_527759	217.58	0.420	0.000	0.479	0.408	0.421
4541	wsnp_Ex_c65790_64067038	217.58	0.401	0.000	0.465	0.408	0.401
5741	wsnp_Ex_rep_c73919_71799491	217.58	0.434	0.000	0.488	0.490	0.426
7312	wsnp_Ku_c8088_13805885	217.58	0.440	0.000	0.491	0.480	0.435
4822	wsnp_Ex_c8641_14485630	217.58	0.494	0.000	0.483	0.490	0.491
5090	wsnp_Ex_rep_c103248_88252209	217.58	0.492	0.000	0.488	0.490	0.488
2972	wsnp_Ex_c25438_34703568	217.58	0.633	0.000	0.461	0.408	0.643

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
5260	wsnp_Ex_rep_c66545_64828446	217.58	0.648	0.000	0.488	0.490	0.654
6437	wsnp_Ku_c11848_19265325	218.15	0.491	0.000	0.489	0.472	0.485
5373	wsnp_Ex_rep_c67205_65714295	218.15	0.374	0.000	0.007	0.056	0.396
8359	wsnp_RFL_Contig2598_2253005	221.73	0.494	0.000	0.484	0.496	0.493
1036	wsnp_CAP7_c1453_728076	221.73	0.141	0.000	0.182	0.108	0.144
5128	wsnp_Ex_rep_c105129_89641882	221.73	0.494	0.000	0.484	0.496	0.493
3237	wsnp_Ex_c29445_38480890	228.46	0.000	0.000	0.000	0.000	0.000
1689	wsnp_Ex_c128_254788	228.96	0.141	0.000	0.182	0.108	0.144
1690	wsnp_Ex_c128_255285	228.96	0.436	0.000	0.182	0.108	0.455
3784	wsnp_Ex_c41558_48355943	229.17	0.370	0.000	0.007	0.056	0.393
7019	wsnp_Ku_c4004_7311479	229.78	0.000	0.000	0.000	0.000	0.000
3785	wsnp_Ex_c41558_48356814	230.28	0.005	0.000	0.007	0.056	0.000
5008	wsnp_Ex_rep_c101349_86725007	230.28	0.406	0.000	0.469	0.496	0.381
5846	wsnp_JD_c14405_14144807	233.22	0.373	0.000	0.007	0.056	0.395
2559	wsnp_Ex_c2097_3932976	233.22	0.373	0.000	0.007	0.056	0.395
780	wsnp_CAP11_c5554_2580044	233.73	0.378	0.000	0.021	0.056	0.401
5547	wsnp_Ex_rep_c68587_67434960	233.73	0.498	0.000	0.469	0.496	0.499
4853	wsnp_Ex_c8894_14858193	233.73	0.498	0.000	0.471	0.490	0.499
2253	wsnp_Ex_c17700_26446810	233.73	0.472	0.000	0.296	0.500	0.465
6969	wsnp_Ku_c3780_6950286	233.73	0.498	0.000	0.471	0.490	0.499
778	wsnp_CAP11_c5554_2579815	233.73	0.016	0.000	0.021	0.056	0.012
6869	wsnp_Ku_c3000_5638635	233.73	0.373	0.000	0.007	0.000	0.398
8631	wsnp_Ta_c36_A_1	233.73	0.016	0.000	0.021	0.056	0.012
539	wsnp_BG274019B_Ta_2_1	233.73	0.378	0.000	0.021	0.056	0.401

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
2955	wsnp_Ex_c25217_34481787	233.73	0.374	0.000	0.007	0.056	0.395
5141	wsnp_Ex_rep_c105551_89940311	237.22	0.496	0.000	0.477	0.480	0.497
2511	wsnp_Ex_c20169_29215219	237.22	0.319	0.022	0.386	0.480	0.293
243	wsnp_BE490763B_Ta_2_2	237.22	0.496	0.000	0.477	0.490	0.497
244	wsnp_BE490763B_Ta_2_3	237.22	0.373	0.000	0.007	0.000	0.398
1828	wsnp_Ex_c13908_21774935	237.22	0.373	0.000	0.007	0.000	0.398
8261	wsnp_RFL_Contig1836_982013	237.22	0.496	0.000	0.479	0.451	0.498
242	wsnp_BE490763B_Ta_2_1	237.22	0.415	0.000	0.476	0.490	0.390
68	wsnp_BE406277B_Ta_1_2	237.22	0.325	0.000	0.395	0.490	0.296
4636	wsnp_Ex_c7203_12370983	238.01	0.500	0.000	0.451	0.500	0.500
226	wsnp_BE490267A_Ta_2_1	238.01	0.266	0.000	0.331	0.284	0.264
5939	wsnp_JD_c2273_3105037	238.01	0.386	0.000	0.042	0.000	0.411
1393	wsnp_Ex_c10838_17631243	238.01	0.483	0.000	0.497	0.431	0.471
2294	wsnp_Ex_c18261_27078080	238.01	0.491	0.000	0.374	0.480	0.492
77	wsnp_BE423182B_Ta_2_1	238.01	0.500	0.000	0.448	0.480	0.500
1488	wsnp_Ex_c114_229879	238.01	0.078	0.000	0.102	0.000	0.086
6403	wsnp_Ku_c10640_17548156	238.01	0.005	0.000	0.007	0.000	0.006
6169	wsnp_JD_c744_1111659	238.01	0.188	0.458	0.042	0.000	0.206
1489	wsnp_Ex_c114_230248	238.34	0.344	0.000	0.414	0.490	0.319
6317	wsnp_JD_rep_c67103_42432235	238.34	0.339	0.000	0.409	0.490	0.313
5411	wsnp_Ex_rep_c67543_66165372	239.44	0.328	0.000	0.398	0.408	0.319
1304	wsnp_Ex_c10251_16815404	241.26	0.400	0.000	0.077	0.000	0.424
3035	wsnp_Ex_c26281_35525243	241.26	0.401	0.000	0.083	0.000	0.426
3034	wsnp_Ex_c26281_35524918	241.26	0.401	0.000	0.083	0.000	0.426

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
2189	wsnp_Ex_c17127_25756019	241.26	0.426	0.000	0.152	0.320	0.435
6453	wsnp_Ku_c12037_19549078	241.7	0.440	0.000	0.491	0.480	0.419
5397	wsnp_Ex_rep_c67411_65994109	242.13	0.399	0.000	0.076	0.000	0.423
6047	wsnp_JD_c4243_5350869	242.13	0.370	0.000	0.000	0.000	0.395
2237	wsnp_Ex_c1758_3326792	242.13	0.399	0.000	0.076	0.000	0.423
4275	wsnp_Ex_c55735_58127324	242.13	0.367	0.000	0.436	0.490	0.329
5513	wsnp_Ex_rep_c68194_66973531	242.13	0.399	0.000	0.076	0.000	0.423
2950	wsnp_Ex_c2510_4689918	242.13	0.489	0.000	0.492	0.480	0.490
5512	wsnp_Ex_rep_c68194_66973114	242.13	0.399	0.000	0.076	0.000	0.423
5461	wsnp_Ex_rep_c67840_66538714	242.13	0.053	0.000	0.070	0.000	0.058
3973	wsnp_Ex_c46576_52042185	242.67	0.499	0.000	0.463	0.500	0.499
5789	wsnp_JD_c11975_12326445	243.23	0.440	0.000	0.491	0.480	0.435
2924	wsnp_Ex_c24711_33964543	243.23	0.500	0.000	0.434	0.320	0.500
4256	wsnp_Ex_c54998_57670603	244.76	0.058	0.000	0.076	0.000	0.064
2903	wsnp_Ex_c2445_4573233	246.93	0.442	0.000	0.492	0.451	0.441
5413	wsnp_Ex_rep_c67561_66188180	246.93	0.465	0.000	0.500	0.496	0.460
5414	wsnp_Ex_rep_c67561_66189279	246.93	0.465	0.000	0.500	0.496	0.460
469	wsnp_BF473744B_Ta_2_1	246.93	0.465	0.000	0.500	0.496	0.460
470	wsnp_BF473744B_Ta_2_2	246.93	0.468	0.000	0.500	0.496	0.463
2261	wsnp_Ex_c17845_26604587	246.93	0.654	0.000	0.499	0.496	0.660
4358	wsnp_Ex_c58274_59639258	247.43	0.194	0.000	0.247	0.408	0.164
933	wsnp_CAP12_c197_110707	247.93	0.446	0.000	0.494	0.496	0.437
3935	wsnp_Ex_c4542_8154800	262.44	0.493	0.022	0.216	0.330	0.502
4948	wsnp_Ex_c9628_15927045	269.13	0.476	0.000	0.309	0.467	0.476

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Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
1389	wsnp_Ex_c10796_17575074	269.13	0.476	0.000	0.309	0.467	0.476
5415	wsnp_Ex_rep_c67561_66189356	269.63	0.444	0.000	0.493	0.480	0.439
833	wsnp_CAP11_rep_c4028_1902455	270.48	0.365	0.022	0.431	0.320	0.369
2131	wsnp_Ex_c16425_24923837	270.48	0.479	0.000	0.499	0.480	0.479
6093	wsnp_JD_c52_87219	270.48	0.359	0.000	0.428	0.320	0.363
2130	wsnp_Ex_c16425_24923685	270.48	0.479	0.000	0.499	0.480	0.479
4956	wsnp_Ex_c9729_16071358	270.48	0.411	0.000	0.472	0.320	0.419
7539	wsnp_Ra_c10658_17500389	272.08	0.464	0.366	0.368	0.382	0.470
4356	wsnp_Ex_c58274_59638635	275.11	0.474	0.000	0.305	0.408	0.479
3823	wsnp_Ex_c4230_7639858	275.11	0.495	0.022	0.483	0.431	0.489
3395	wsnp_Ex_c32493_41138957	275.11	0.404	0.000	0.089	0.000	0.428
8478	wsnp_RFL_Contig3522_3685860	275.11	0.490	0.000	0.367	0.500	0.487
4357	wsnp_Ex_c58274_59638884	275.11	0.474	0.000	0.305	0.408	0.479
7850	wsnp_Ra_c31353_40494768	275.11	0.469	0.000	0.287	0.431	0.472
2318	wsnp_Ex_c18503_27349536	275.11	0.068	0.000	0.089	0.000	0.075
8141	wsnp_Ra_rep_c109853_92677055	275.11	0.063	0.000	0.083	0.000	0.070
6050	wsnp_JD_c4343_5462565	278.25	0.499	0.000	0.468	0.496	0.498
8195	wsnp_Ra_rep_c74497_72390803	278.25	0.406	0.000	0.469	0.496	0.390
5145	wsnp_Ex_rep_c105744_90067453	278.83	0.315	0.000	0.385	0.382	0.308
3741	wsnp_Ex_c40976_47910144	278.83	0.315	0.000	0.385	0.382	0.308
3742	wsnp_Ex_c40976_47910672	278.83	0.315	0.000	0.385	0.382	0.308
4294	wsnp_Ex_c5619_9884202	279.98	0.298	0.000	0.367	0.320	0.296
5958	wsnp_JD_c2512_3403544	279.98	0.016	0.000	0.021	0.000	0.018
7615	wsnp_Ra_c13679_21569624	279.98	0.302	0.000	0.370	0.382	0.292

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Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
1708	wsnp_Ex_c12922_20473104	279.98	0.298	0.000	0.367	0.382	0.288
6054	wsnp_JD_c43812_30492711	279.98	0.302	0.000	0.370	0.382	0.292
1707	wsnp_Ex_c12922_20472434	279.98	0.496	0.000	0.477	0.496	0.495
2179	wsnp_Ex_c16948_25537916	283.72	0.476	0.000	0.499	0.500	0.471
4866	wsnp_Ex_c9025_15039930	283.72	0.477	0.000	0.499	0.496	0.474
2701	wsnp_Ex_c22271_31463382	285.21	0.420	0.000	0.479	0.451	0.416
2379	wsnp_Ex_c1915_3618286	285.21	0.420	0.000	0.479	0.451	0.416
4909	wsnp_Ex_c942_1806632	285.21	0.461	0.000	0.499	0.496	0.449
2678	wsnp_Ex_c2203_4130096	286.72	0.472	0.000	0.500	0.500	0.465
5177	wsnp_Ex_rep_c110284_92725051	287.22	0.037	0.000	0.049	0.202	0.018
4098	wsnp_Ex_c5123_9089624	287.97	0.476	0.000	0.499	0.480	0.475
2158	wsnp_Ex_c16627_25162803	287.97	0.461	0.000	0.499	0.480	0.459
4900	wsnp_Ex_c9345_15516291	287.97	0.038	0.000	0.049	0.202	0.018
2676	wsnp_Ex_c2203_4129271	291.52	0.473	0.000	0.500	0.500	0.466
2677	wsnp_Ex_c2203_4129457	291.52	0.466	0.000	0.500	0.500	0.460
7652	wsnp_Ra_c16333_24961476	292.04	0.404	0.000	0.467	0.467	0.395
5051	wsnp_Ex_rep_c102127_87360034	292.04	0.449	0.000	0.221	0.202	0.463
4095	wsnp_Ex_c5123_9087869	292.04	0.464	0.000	0.499	0.480	0.449
1291	wsnp_Ex_c1016_1943126	292.04	0.173	0.000	0.221	0.202	0.169
4097	wsnp_Ex_c5123_9089025	292.04	0.464	0.000	0.499	0.480	0.449
1273	wsnp_Ex_c10071_16554911	292.04	0.005	0.000	0.007	0.000	0.006
1293	wsnp_Ex_c1016_1943827	292.04	0.173	0.000	0.221	0.202	0.169
7371	wsnp_Ku_c9901_16493072	292.04	0.655	0.000	0.499	0.480	0.660
3075	wsnp_Ex_c26818_36041748	292.54	0.078	0.000	0.102	0.202	0.064

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
3176	wsnp_Ex_c28627_37743031	294.28	0.634	0.000	0.463	0.408	0.645
2873	wsnp_Ex_c24135_33382521	294.49	0.420	0.000	0.479	0.451	0.416
1765	wsnp_Ex_c13353_21046550	296.08	0.315	0.000	0.385	0.157	0.329
2459	wsnp_Ex_c19772_28771627	296.08	0.312	0.000	0.381	0.202	0.322
638	wsnp_BQ172173B_Ta_2_2	296.66	0.377	0.000	0.014	0.000	0.402
8295	wsnp_RFL_Contig2123_1397739	299.03	0.378	0.000	0.446	0.245	0.390
7620	wsnp_Ra_c13963_21949302	299.03	0.304	0.000	0.373	0.245	0.310
6122	wsnp_JD_c6010_7167159	300.37	0.302	0.000	0.370	0.245	0.308
6121	wsnp_JD_c6010_7167084	300.37	0.302	0.000	0.370	0.245	0.308
4890	wsnp_Ex_c922_1775246	303.52	0.489	0.000	0.363	0.480	0.490
7279	wsnp_Ku_c7297_12596001	303.68	0.000	0.000	0.000	0.000	0.000
2875	wsnp_Ex_c24135_33382813	303.68	0.207	0.000	0.262	0.451	0.170
2872	wsnp_Ex_c24135_33382318	303.68	0.483	0.000	0.497	0.467	0.474
3175	wsnp_Ex_c28627_37742718	303.68	0.413	0.000	0.474	0.408	0.414
3509	wsnp_Ex_c3506_6414757	303.68	0.000	0.000	0.000	0.000	0.000
6561	wsnp_Ku_c15498_24122936	303.68	0.653	0.000	0.497	0.467	0.660
2874	wsnp_Ex_c24135_33382700	304.01	0.453	0.000	0.497	0.467	0.451
2215	wsnp_Ex_c174_340829	305.3	0.000	0.000	0.000	0.000	0.000
7909	wsnp_Ra_c3955_7262354	306.15	0.499	0.000	0.428	0.500	0.499
2502	wsnp_Ex_c20029_29064315	306.15	0.474	0.000	0.500	0.490	0.472
7640	wsnp_Ra_c15621_24073604	306.15	0.117	0.000	0.152	0.056	0.124
1076	wsnp_CAP7_c317_172502	306.59	0.302	0.000	0.370	0.202	0.311
8029	wsnp_Ra_c62106_62286811	307.26	0.492	0.000	0.487	0.382	0.496
8145	wsnp_Ra_rep_c111598_93800332	307.26	0.500	0.000	0.448	0.496	0.500

Appendix A Continued

Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
3937	wsnp_Ex_c45468_51254832	307.48	0.391	0.000	0.457	0.480	0.378
3938	wsnp_Ex_c45468_51254978	307.48	0.000	0.000	0.000	0.000	0.000
8406	wsnp_RFL_Contig2914_2757372	308.61	0.259	0.000	0.323	0.382	0.243
8266	wsnp_RFL_Contig1892_1042675	308.61	0.462	0.000	0.499	0.451	0.463
5460	wsnp_Ex_rep_c67838_66536117	309.76	0.500	0.043	0.458	0.480	0.499
1822	wsnp_Ex_c13865_21720466	313.6	0.492	0.043	0.490	0.496	0.489
8534	wsnp_RFL_Contig3917_4326857	313.6	0.299	0.000	0.368	0.484	0.268
3714	wsnp_Ex_c4024_7278036	315.62	0.248	0.000	0.310	0.472	0.212
1599	wsnp_Ex_c12223_19533198	315.83	0.499	0.044	0.471	0.490	0.498
3010	wsnp_Ex_c25943_35203857	315.83	0.499	0.043	0.471	0.490	0.497
5809	wsnp_JD_c12346_12606967	316.4	0.499	0.085	0.441	0.493	0.499
5810	wsnp_JD_c12346_12607102	316.4	0.333	0.022	0.399	0.493	0.289
1324	wsnp_Ex_c10441_17078853	317.74	0.580	0.458	0.608	0.415	0.588
7112	wsnp_Ku_c48694_54811376	320.28	0.190	0.022	0.237	0.111	0.198
7955	wsnp_Ra_c4660_8405634	327.93	0.322	0.000	0.392	0.245	0.329
5024	wsnp_Ex_rep_c101581_86924089	327.93	0.322	0.000	0.392	0.245	0.329
7626	wsnp_Ra_c14267_22357509	328.25	0.164	0.022	0.204	0.451	0.119
2343	wsnp_Ex_c18686_27560445	328.52	0.108	0.022	0.134	0.251	0.092
8589	wsnp_RFL_Contig4402_5154408	334.58	0.459	0.454	0.460	0.408	0.463
5081	wsnp_Ex_rep_c103064_88104690	335.19	0.444	0.064	0.490	0.451	0.443
571	wsnp_BG605258B_Ta_2_7	335.19	0.468	0.483	0.462	0.408	0.473
570	wsnp_BG605258B_Ta_2_1	350.12	0.216	0.022	0.269	0.208	0.217
5007	wsnp_Ex_rep_c101342_86720058	350.57	0.437	0.494	0.405	0.157	0.453
5738	wsnp_Ex_rep_c72569_70908990	362.01	0.491	0.498	0.481	0.498	0.489

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Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
6600	wsnp_Ku_c16547_25454123	363.51	0.498	0.495	0.499	0.108	0.500
1348	wsnp_Ex_c10555_17235832	366.25	0.498	0.491	0.492	0.056	0.499
3596	wsnp_Ex_c3695_6740339	368.17	0.495	0.495	0.486	0.000	0.500
7671	wsnp_Ra_c17622_26522072	369.6	0.028	0.000	0.037	0.000	0.031
1535	wsnp_Ex_c11758_18896434	370.46	0.236	0.416	0.158	0.000	0.257
6656	wsnp_Ku_c18587_27915541	370.46	0.240	0.423	0.158	0.000	0.261
3474	wsnp_Ex_c34303_42642389	370.99	0.500	0.482	0.499	0.496	0.500
746	wsnp_CAP11_c3226_1588070	378.06	0.500	0.498	0.500	0.496	0.499
8504	wsnp_RFL_Contig3712_3953814	378.7	0.368	0.143	0.418	0.496	0.334
2377	wsnp_Ex_c19094_28015035	379.57	0.423	0.434	0.419	0.451	0.420
5093	wsnp_Ex_rep_c103381_88353000	379.57	0.500	0.469	0.497	0.245	0.497
692	wsnp_CAP11_c1820_985143	379.57	0.005	0.022	0.000	0.000	0.006
3252	wsnp_Ex_c298_580660	380.82	0.377	0.358	0.383	0.472	0.363
2509	wsnp_Ex_c20155_29202599	381.82	0.011	0.000	0.014	0.056	0.006
5442	wsnp_Ex_rep_c67697_66363222	385.45	0.355	0.449	0.315	0.360	0.355
4118	wsnp_Ex_c5193_9204522	385.45	0.027	0.043	0.021	0.000	0.030
3773	wsnp_Ex_c41300_48154348	385.45	0.027	0.043	0.021	0.000	0.030
8018	wsnp_Ra_c58860_60407020	387.23	0.367	0.295	0.387	0.451	0.355
1667	wsnp_Ex_c12675_20144479	388.04	0.358	0.280	0.380	0.451	0.345
1668	wsnp_Ex_c12675_20144755	388.04	0.016	0.022	0.014	0.000	0.018
2046	wsnp_Ex_c15646_23969140	388.04	0.435	0.351	0.454	0.327	0.443
7456	wsnp_Ku_rep_c69632_69044288	388.04	0.318	0.456	0.254	0.000	0.343
2551	wsnp_Ex_c2066_3877373	389.89	0.646	0.621	0.651	0.633	0.637
4619	wsnp_Ex_c707_1391630	389.89	0.257	0.281	0.249	0.108	0.271

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Index	Marker	Position	All	*Sr36*	No *Sr36*	Before 1950	After 1950
3315	wsnp_Ex_c31064_39902843	393.39	0.438	0.449	0.432	0.480	0.433
2094	wsnp_Ex_c16074_24502385	394.04	0.609	0.521	0.619	0.647	0.602
6164	wsnp_JD_c7305_8404286	395.28	0.636	0.656	0.607	0.578	0.641
6852	wsnp_Ku_c28820_38731137	395.92	0.346	0.372	0.337	0.320	0.349
5694	wsnp_Ex_rep_c70525_69448648	395.92	0.335	0.361	0.326	0.251	0.343
2946	wsnp_Ex_c25043_34305764	395.92	0.496	0.380	0.499	0.500	0.494
-	*TaSus2-2B*	-	0.409	0.000	0.080	0.000	0.434
-	TaPpdBJ001	-	0.446	0.541	0.115	0.000	0.478
-	TaPpdBJ003	-	0.107	0.000	0.140	0.061	0.111