further investigate the events that lead to this fusion, we coupled a protocol for genome complexity reduction with the Illumina platform, to sequence at 40X coverage a bulk of seven RH lines carrying or missing the *scs* gene. The sequences identified in the first (positive) bulk but not in the second (negative), were considered to represent the *scs* region. These 3.2 K differential pair-ends sequences were then extended to 1–5 Kb in size, employing the publicly available 50X survey sequences of *Aegilops tauschii*. Furthermotr, these extended sequences were assembled with DNAStar into 556 contigs spanning 1.2 Mb with an N50 of 3Kb. This gapped sequence was then anchored to the scaffold RH map, pin-pointing the *scs* locus to an interval of just eight genes. The result of coupling Illumina sequencing, Wheat Zapper synteny analysis, and RH populations is discussed here in relation to the evolutionary importance of the *scs* region and its map based cloning.

Identification of a candidate barley stem rust susceptibility gene determining the recessive nature of Rpg4-mediated Ug99 resistance in barley.

Deepika Arora [1], Xue Wang [1], Patrick Gross [1], Brian Steffenson [2], and Robert Brueggeman [1].

[1] Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA and [2] Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108-6030, USA.

The *rpg4*/*Rpg5* locus in barley (*Hordeum vulgare*) provides recessive resistance against several wheat stem rust races (*Puccinia graminis* f. sp. *tritici*) including QCCJ and the highly virulent race TTKSK (aka Ug99). Three genes required for wheat stem rust resistance (*HvADF3*, *Rpg5*, and *HvRGA1*) were identified in the ~70kbp *rpg4*/*Rpg5* stem rust resistance locus using high-resolution mapping and virus induced gene silencing. The dominant rye stem rust resistance gene *Rpg5* is predicted to have the typical R-gene domains including the nucleotide-binding site (NBS), leucine rich repeat (LRR), and serine/ threonine protein kinase (STPK) domains. The *Rpg5* gene appears to condition compatible or incompatible interactions with the wheat stem rust races QCCJ and Ug99, because it is the only polymorphic gene correlating with resistance and susceptibility in the delimited *rpg4*/*Rpg5* region. Sequence analysis of *rpg5* susceptible alleles showed that they make up two groups. The group-1 susceptible lines contain an insertion/deletion region having a predicted functional protein phosphatase 2C gene (*HvPP2C*) in place of the *Rpg5* STPK domain (Harrington, Steptoe, and Sm89010). The group-2 susceptible lines have an intact STPK domain but a predicted nonfunctional *rpg5* allele due to a single cytosine insertion causing a frame shift mutation resulting in a premature stop codon (Golden Promise, OSU6, and MD2). Analysis of F_2 progeny from crosses between Q21861 and group-1 and -2 susceptible lines segregated in 1:3 ratio (resistant:susceptible) when the *HvPP2C* gene is present but in a 3:1 ratio (resistant:susceptible) when the *HvPP2C* gene is absent. Thus, it appears that the previously identified *Rpg5* dominant rye stem rust resistance gene also imparts *rpg4*-mediated wheat stem rust resistance and behaves as a dominant gene in the absence of the *HvPP2C* gene but as a recessive resistance gene in the presence of *HvPP2C*. The data suggests that components of *rpg4* and *Rpg5* resistance are not distinct and the difference in the dominant or recessive nature of resistance is due to the *HvPP2C* gene acting as a dominant susceptibility factor that suppresses *rpg4*/*Rpg5*-mediated resistance against the wheat stem rust races including Ug99.

Targeted re-sequencing of the wheat exome and the generation of public co-dominant single nucleotide polymorphism markers.

Alexandra M. Allen [1], Gary L.A. Barker [1], Simon Griffiths [2], Cristobal Uauy [2], Peter Jack [3], Simon Berry [4], Peter Werner [5], James P. E. Melichar [6], Jane Coghill [1], Mark Winfield [1], Paul Wilkinson [1], Amanda Burridge [1], Jane McDougall [7], Rhian Gwilliam [7], Phil Robinson [7], and Keith J Edwards [1].

[1] School of Biological Sciences, University of Bristol, Bristol, UK; [2] John Innes Centre, Norwich, UK; [3] RAGT, Ickleton, Essex, UK; [4] Limagrain, Woolpit, Suffolk, UK; [5] KWS, Thriplow, Hertfordshire, UK; [6] Syngenta Seeds Ltd, Hill Farm Road, Whittlesford, Cambridge, UK; and [7] KBioscience, Unit 7, Maple Park, Hertfordshire, UK.

The complex nature of the wheat genome has, until recently, resulted in a lack of single nucleotide polymorphism (SNP)-based molecular markers of practical use to wheat breeders. Recently, large numbers of SNP-based wheat markers have been made available via the use of next generation sequencing combined with a variety of genotyping platforms. However, many of these markers and platforms have difficulty distinguishing between heterozygote and homozygote indi-

viduals and are therefore of limited use to wheat breeders carrying our commercial scale breeding programs. To identify co-dominant SNP-based markers, which are capable of distinguishing between heterozygotes and homozygotes, we have used targeted re-sequencing of the wheat exome to generate large amounts of genic sequences from eight varieties. Using a bioinformatics approach, these sequences have been used to identify 95,266 putative, gene-based, single nucleotide polymorphisms, of which 10,251 were classified as being suitable markers for the discrimination of homozygote and heterozygote individuals. Validation of a sample of these markers confirmed that 81% could easily discriminate between heterozygous and homozygous individuals. Comparison of these co-dominant markers with dominant markers indicated that both marker types were distributed similarly across genetic maps. In addition, the use of both marker types across two U.K. mapping populations revealed that the two populations had different levels of polymorphism across the A, B, and D genomes. The new co-dominant markers described here are capable of complete genotypic classification of a segregating locus in polyploid wheat and can be used on a variety of genotyping platforms; as such they represent a powerful tool for wheat breeders. The markers and related information described here have been made publically available on an interactive web-based database in order to facilitate their use in genotyping programs worldwide.

Molecular adaptation to cooler climates and ecological diversification of Pooideae.

Magnus D. Vigeland [1], Manuel Spannagl [2], Torben Asp [3], Cristiana Paina [3], Heidi Rudi [4], Odd-Arne Rognli [4], Siri Fjellheim [4], and **Simen R. Sandve** [4].

[1] Department of Medical Genetics, Oslo University Hospital and University of Oslo, Oslo, Norway; [2] Helmholtz Zentrum München, Institute of Bioinformatics and Systems Biology, München, Germany; [3] Department of Genetics and Biotechnology, Faculty of Agricultural Sciences, Research Centre Flakkebjerg, Aarhus University, Denmark; and [4] Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, Aas, Norway.

Adaptation to temperate environments is a common feature in the grass subfamily Pooideae, suggesting an ancestral origin of low temperature stress tolerance dating back to the beginning of Pooideae taxonomic divergence. It also has been suggested that climate cooling during the Eocene-Oligocene transition (~34MYA) was important for cold climate adaptation in the core Pooideae clade, in which the Triticeae species is contained. Here we analyze the molecular evolution of genes involved in low-temperature stress response and present evidence for the importance these genes in the evolution of the Pooideae lineage.

Maximum likelihood-based phylogenetic methods were used to estimate substitution rates in Pooideae species relative to rice and test the hypothesis that cold induced loci were under positive selection during radiation of the Pooideae lineage. In addition we carried out in depth studies of the evolution of three Pooideae-specific gene families, *CBF*, *FST*, and *IRIP* genes, known to be central in core Pooideae low temperature stress responses.

Phylogenies of 4330 orthologous loci were produced, of which 388 loci were defined as low-temperature induced in Pooideae. A general increase in substitution rates was observed for all genes in the Pooideae lineage relative to rice, and this rate increase was higher for nonsynonymous substitutions (+7–20%) compared to synonymous substitutions (+0–7%). However, the nonsynonymous substitution rate increase in Pooideae was significantly higher in those loci defined as low-temperature induced. Tests for positive selection on the basal Pooideae branch showed a 3.3-fold increase in significant tests for low temperature induced loci compared to all loci. Analyses of Pooideae-specific gene families involved in low temperature stress responses identified both ancient evolutionary innovations (basal Pooideae), as well as more recent innovations in carbohydrate metabolism specific for core Pooideae.

The Pooideae lineage evolved from a tropical/subtropical ancestor to become a taxonomic group with ecological dominance in temperate ecosystems. Our results suggest that adaptive evolution of low temperature responses was of importance in the basal Pooideae, possibly enabling the ecological radiation into cooler ecosystems.