

Wild sex in the grasses

Jason A. Able¹ and Peter Langridge²

¹Molecular Plant Breeding Cooperative Research Centre, School of Agriculture, Food & Wine, The University of Adelaide, Glen Osmond, SA 5064, Australia

²Australian Centre for Plant Functional Genomics, School of Agriculture, Food & Wine, The University of Adelaide, Glen Osmond, SA 5064, Australia

To date, alien introgression of agronomically important traits into bread wheat (*Triticum aestivum*) from wild relatives has not been readily achievable through traditional breeding practices. However, this door might now be unlocked. The insightful research published recently by Graham Moore and his team delivers a likely candidate in the form of a *cdc2*-kinase-related gene family for the *Ph1* locus – a chromatin region located on chromosome 5B that is responsible for homologous chromosome pairing integrity in bread wheat.

Exploiting mutants in breeding programmes

With the world's population predicted to be more than 9 billion by the year 2050, we as a society at large must recognize the importance of agriculture and its impact on feeding this ever-expanding population base. Although plant breeding programmes have intensified, not just in developed countries but also globally, we have witnessed a steady decline in the genetic diversity that is available for sustained improvement of agronomic traits such as yield. One way of increasing diversity is through the use of induced mutations – cereal plant breeders have had both X-ray and chemically induced mutants at their disposal since the late 1950s. Recent work with Targeting Induced Local Lesions IN Genomes (TILLING) [1] has revitalized the mutation breeding area. However, there is also enormous diversity in wheat landraces and wild relatives. It is still not clear just how much of the natural variability within the *Triticeae* family has been harnessed: in bread wheat, there are predictions that it might be as little as 10–15% of the available gene pool. To reverse this 'selection bottleneck' and to replenish the germplasm pool while maintaining the attributes selected by breeders over the past century, we must utilize technologies that allow introgression of alien chromatin and enable selection of novel alleles without sacrificing important traits such as quality. Since the discovery of the *Ph* (pairing homoeologous) (see Glossary) mutants in bread wheat [2–5], there has been a major effort to understand the cytological genetic behaviour and molecular basis of such mutants and their wild-type genes [6].

Wheat genetics and meiosis

Bread wheat is a polyploid, specifically an allohexaploid containing three genomes named A, B and D, each derived

from a different progenitor species. There were two polyploidization events that led to bread wheat. The first created the tetraploid wheats (the durum or pasta wheats), which are important crops in their own right; the second added a third genome from goat grass (*Aegilops tauschii*) to form the hexaploid bread wheats. Despite having three genomes, bread wheat behaves as a diploid during meiosis with only homologous chromosomes pairing with one another (Figure 1a).

Meiosis is an obligatory process undertaken by all sexually reproducing organisms that comprises one round of DNA replication followed by two rounds of cell division. During the first round of cell division in bread wheat, typically no homoeologous chromosome pairing occurs (Figure 1b), and of the recombination that does take place, this is restricted to homologous chromosomes only. This barrier diminishes the breeder's ability to generate more variability through unlocking and harnessing the wealth of agronomically important traits that are within some of the more distant relatives of bread wheat. The underlying reason as to why this lack of potential diversity in the bread wheat gene pool exists is due to the *Ph1* pairing mechanism preventing such combinations from occurring. Importantly, in the absence of this locus (and other *Ph* loci in general), homoeologous chromosome pairing interactions can and do occur [7]. Another gene locus responsible for homoeologous associations within the bread wheat genome is the *Ph2* locus. Although progress has been made delineating this region in recent times [8], further research still needs to be undertaken to ascertain the gene(s) responsible for this phenotype. Significantly, bread wheat is not the only allopolyploid where such pairing control genes exist. Oats (*Avena sativa*) and the *Lolium–Festuca* complex are further

Glossary

Allohexaploid: an organism that contains three genomes, each of them being derived from a different progenitor species.

Heterochromatin: chromatin that is predominantly in a condensed form and therefore inactive.

Homologous chromosome pairing: pairing of chromosomes that are identical with respect to both gene content and order.

Homoeologous chromosome pairing: pairing of chromosomes that are similar with respect to both gene content and order (differing in their repetitive DNA content).

Homologous recombination: the exchange of DNA molecules between paired chromosomes during prophase I of meiosis.

***Ph* loci:** regions of the bread wheat genome associated with suppressing homoeologous or promoting homologous chromosome pairing.

Polyploidization: the creation or formation of a nucleus that contains more than two sets of chromosomes.

Corresponding author: Able, J.A. (jason.able@adelaide.edu.au).

Available online 11 May 2006

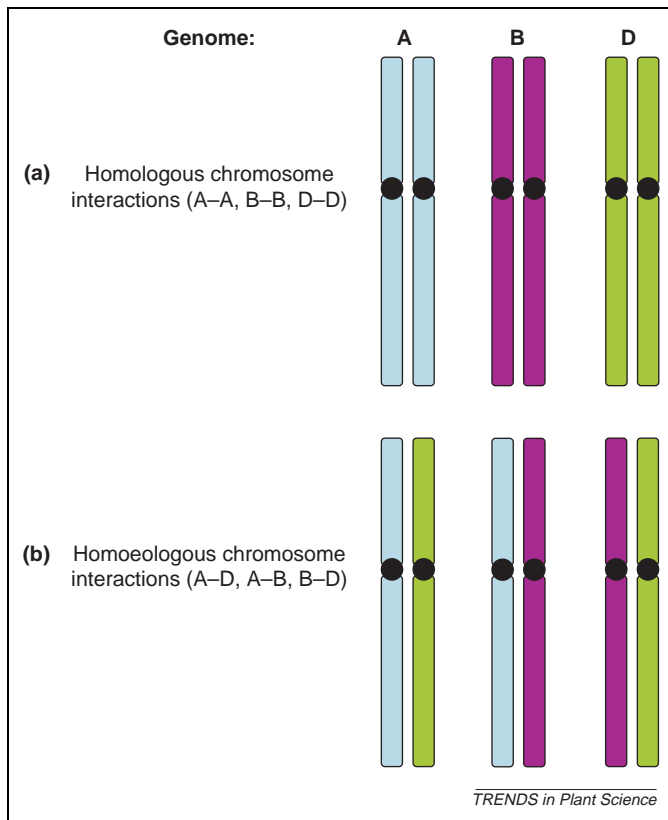


Figure 1. (a) Homologous and (b) homoeologous chromosome pairing in bread wheat. For all seven bread wheat chromosome groups, homologous chromosomes will pair (e.g. 1A–1A, 2A–2A) at meiosis but homoeologous chromosomes will not pair with one another.

examples of where there are pairing control mechanisms in place that have not yet been deciphered. Even so, by manipulating these breeding barriers, particularly in wheat, one can envisage crop improvement taking a revolutionary step forward, similar to that of Norman Borlaug's Green Revolution in the 1960s.

The importance of polyploidy in shaping genome structure

Although there have been various models and hypotheses [9] proposed to account for the origin and evolution of genetic control of chromosome pairing in polyploids, to date there is no direct evidence to suggest that any are correct. Given that bread wheat is a hexaploid and that the genome size is one of the largest in higher eukaryotes, it makes the task of deciphering the structure of this complex organism that much more difficult than for a simpler diploid genome such as rice (albeit a possible ancient tetraploid [10]). Nonetheless, with the gradual molecular refinement of loci such as *Ph1*, scientists will be better equipped to understand the complexity of this genome. Put in an evolutionary context, polyploidy has played a major role in shaping the development of crop plants that are grown today. Through gene duplication and the subsequent redundancy in homoeologous gene function, gene diversification has given rise (in some cases) to genes with novel functions [11]. The research conducted by Graham Moore and colleagues suggests that the *Ph1* locus arose after wheat polyploidization [12].

What lies ahead?

Unravelling the complexities of the *Ph1* locus and its importance for wide-crosses to relatives of bread wheat has been the research focus of Moore's group for more than 10 years. To dissect the vast amount of data that Moore and colleagues have generated and reported [12], verification of the *cdc2* candidates and/or heterochromatic insertion as *Ph1* is likely to require the generation and analysis of transgenic wheat lines with the *cdc2* cluster knocked out from chromosome 5B. Because a targeted transformation system for wheat might still be sometime off, the best approach at present would be analysing knock down lines for each of the *cdc2* candidates reported, independently and in combination. If *cdc2*-related kinases are responsible for the *Ph1* phenotype, it is likely that they (or possibly a single *cdc2* gene within the cluster) have diverged since polyploidization and/or after the insertion of the sub-telomeric heterochromatic region (as Moore and his team suggest) because the majority of *cdc2*-related kinases are commonly associated with mitotic events as opposed to meiotic events [13–15].

An immediate objective and extension of the existing research will be to identify deletion derivatives in the *Ph1* region. These mutants would consist of various combinations of the *cdc2* gene cluster, with or without the heterochromatic block. Mutants such as these could then be used by Moore and his team to relate specific segments to the *Ph1* pairing phenotype. If it were found that the absence of only one or two of the *cdc2* gene candidates produced a full *Ph1* pairing phenotype, then the benefit of such an approach, although tedious, would reduce the number of gene constructs needed for transgenic research.

Although Moore and colleagues have reported significant advances [12], many unanswered questions regarding *Ph1* (and other *Ph* loci) remain. Specifically, what is the mechanism of action for *Ph1*? Although descriptive hypotheses have been put forward [16], the mechanism is still unclear. A more detailed explanation of the mechanism will be needed before the power of these loci can be unleashed as a highly controlled tool for alien gene introgression in bread wheat. When this happens, the vast pool of untapped variation in wild wheat relatives will become available for breeding programmes. Gregor 'Johann' Mendel – the father of genetics – would no doubt have been impressed with such advancements if he were with us today.

References

- 1 McCallum, C.M. *et al.* (2000) Targeted screening for induced mutations. *Nat. Biotechnol.* 18, 455–457
- 2 Riley, R. and Chapman, V. (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* 182, 713–715
- 3 Mello-Sampayo, T. (1971) Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. *Nat. New Biol.* 230, 22–23
- 4 Sears, E.R. (1977) An induced mutant with homoeologous pairing in common wheat. *Can. J. Genet. Cytol.* 19, 585–593
- 5 Wall, A.M. *et al.* (1971) The position of a locus on chromosome 5B of *Triticum aestivum* affecting homoeologous meiotic pairing. *Genet. Res.* 18, 329–339
- 6 Martinez, M. *et al.* (2001) The *Ph1* and *Ph2* loci play different roles in the synaptic behaviour of hexaploid wheat *Triticum aestivum*. *Theor. Appl. Genet.* 103, 398–405

- 7 Sears, E.R. (1976) Genetic control of chromosome pairing in wheat. *Annu. Rev. Genet.* 10, 31–51
- 8 Sutton, T. *et al.* (2003) The *Ph2* pairing homoeologous locus of wheat (*Triticum aestivum*): identification of candidate meiotic genes using a comparative genetics approach. *Plant J.* 36, 443–456
- 9 Jenczewski, E. and Alix, K. (2004) From diploids to allopolyploids: the emergence of efficient pairing control genes in plants. *Crit. Rev. Plant Sci.* 23, 21–45
- 10 Goff, S.A. *et al.* (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296, 92–100
- 11 Wendel, J.F. (2000) Genome evolution in polyploids. *Plant Mol. Biol.* 42, 225–249
- 12 Griffiths, S. *et al.* (2006) Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. *Nature* 439, 749–752
- 13 Nasmyth, K. and Nurse, P. (1981) Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* 182, 119–124
- 14 Nurse, P. and Bissett, Y. (1981) Gene required in G_1 for commitment to cell cycle and in G_2 for control of mitosis in fission yeast. *Nature* 292, 558–560
- 15 Escargueil, A.E. *et al.* (2001) Recruitment of cdc2 kinase by DNA topoisomerase II is coupled to chromatin remodeling. *FASEB J.* 15, 2288–2290
- 16 Moore, G. (2002) Meiosis in allopolyploids – the importance of ‘Teflon’ chromosomes. *Trends Genet.* 18, 456–463

1360-1385/\$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tplants.2006.04.004

Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface

Michel Chalot, Damien Blaudez and Annick Brun

Université Henri Poincaré, Nancy I, Faculté des Sciences et Techniques, IFR 110 Génomique, Ecophysiologie et Ecologie fonctionnelles, UMR INRA-UHP 1136 “Interactions Arbres/Micro-organismes”, BP 239, 54506 Vandoeuvre-les-Nancy Cedex, France

In mycorrhizal associations, the fungal partner assists its plant host with nitrogen and phosphorus uptake while obtaining photosynthetically fixed carbon. Recent studies in mycorrhiza have highlighted the potential for direct transfer of ammonia from fungal to plant cells. This presents a new perspective on nitrogen transfer at the mycorrhizal interface, which is discussed here in light of recent progress made in characterizing a large array of membrane proteins that could fulfil the function of transporting ammonia.

Nitrogen transfer at the mycorrhizal interface: a new face to an old question?

Under natural conditions, the majority of plants are believed to have mycorrhizal associations (intimate symbiotic association between fungi and roots) with either arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi. This status relies on the bidirectional transfer of nutrients between the two symbionts, which was first demonstrated by Elias Melin and Harald Nilsson >50 years ago [1]. However, the identity of the N solutes (organic versus inorganic) translocated by mycorrhizal fungi and whether or not fungal symbionts translocate a significant part of the newly absorbed or formed ammonia* are questions that have remained unanswered until recently. In both AM and ECM fungi, the bulk of evidence in support of organic N transfer was derived primarily from ^{15}N tracer experiments and enzymatic studies

(reviewed in Refs [1,2]). Experimental observations have indicated that glutamine (in ECM fungi) or arginine (in AM fungi) are usually the principal nitrogenous products accumulated during periods of ammonium feeding at the uptake site, providing support for the importance of these amino acids in N transfer between fungal and plant cells. This is the ‘traditional view’, as depicted in Figure 1. Rapid assimilation of inorganic N at the uptake site can ensure that internal demands are satisfied before transfer to the host plant and can also prevent toxic accumulation of ammonium ions. Recently, Philip Pfeffer and collaborators [3,4] reported that beneficial AM fungi transfer substantial amounts of nitrogen to their plant hosts, probably as ammonia. Uwe Nehls’ research group have also recently suggested that ammonia could be transferred directly in an ECM association [5], which reinforces this model. The elucidation of this elegant mechanism raised puzzling questions about the mechanisms involved in the transfer of ammonia and the consequences of this model on the N metabolism of the two partners. Here, we will focus on N transfer at the symbiotic interface rather than N uptake from the external fungal phase (i.e. in soil).

Requirements for efficient ammonia transfer between fungus and plant

The arginine-derived origin of ammonia in AM symbiosis has been demonstrated [6] but the origin of ammonia in ECM symbiosis is less clear given the high capacities of fungal cells to assimilate ammonia [1,2]. Low assimilatory capacities of intraradical fungal cells would be needed to sustain ammonia transfer: this is indeed supported by a strong down-regulation of the gene coding the ammonium

Corresponding author: Chalot, M. (michel.chalot@scbiol.uhp-nancy.fr).

Available online 11 May 2006

* NH_3 refers to molecular ammonia, NH_4^+ to ammonium ions and ammonia to the sum of both.