Techniques & Applications

Heterotic Haplotype Capture: precision breeding for hybrid performance

Rod J. Snowdon[1], **Amine Abbadi**[2], **Tobias Kox**[3], **Thomas Schmutzer**[4], **and Gunhild Leckband**[5]

[1] Department of Plant Breeding, IFZ Research Centre for Biosystems, Land Use, and Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26–32, 35392 Giessen, Germany
[2] NPZ Innovation GmbH, Hohenlieth-Hof, 24363 Holtsee, Germany
[3] Institute of Crop Science and Resource Conservation (INRES), Katzenburgweg 5, 53115 Bonn, Germany
[4] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Correnstrasse 3, 06466 Gatersleben, Germany
[5] German Seed Alliance GmbH, Hohenlieth 24363, Germany

The need to improve hybrid performance, abiotic stress tolerance, and disease resistance without compromising seed quality makes the targeted capture of untapped diversity a major objective for crop breeders. Here we introduce the concept of Heterotic Haplotype Capture (HHC), in which genome sequence imputation is used to trace novel heterozygous chromosome blocks contributing to hybrid performance in large, structured populations of interrelated F_1 hybrids containing interesting new diversity for breeding.

The ups and downs of heterotic potential

Directed exploitation of heterosis (see Glossary), achieved through breeding of hybrid varieties from crosses between genetically divergent gene pools, has been one of the most important contributors to yield increases in major crops over recent decades. By contrast, strong selection during domestication and breeding reduces genetic diversity in breeding pools, consequently causing erosion of heterotic potential. This 'breeders' paradox' finds a prime example in *Brassica napus* (oilseed rape, canola) a globally important oilseed crop with a recent domestication history [1] and extreme genetic bottlenecks created by stringent selection for specific seed qualities within ecogeographically divergent international breeding pools [2]. High-throughput sequencing technologies and high-density genotyping improve the prospects of characterising and overcoming such bottlenecks and increasing yield gain through precision breeding. With genome sequences and phenotype information from founder accessions, the capture of heterotic haplotypes from large, comprehensively genotyped test populations, in combination with phenotyped F_1 hybrids segregating for completely new diversity, provide a powerful platform for predictive breeding towards elevated hybrid performance.

Capturing novel diversity in F_1 test hybrid populations

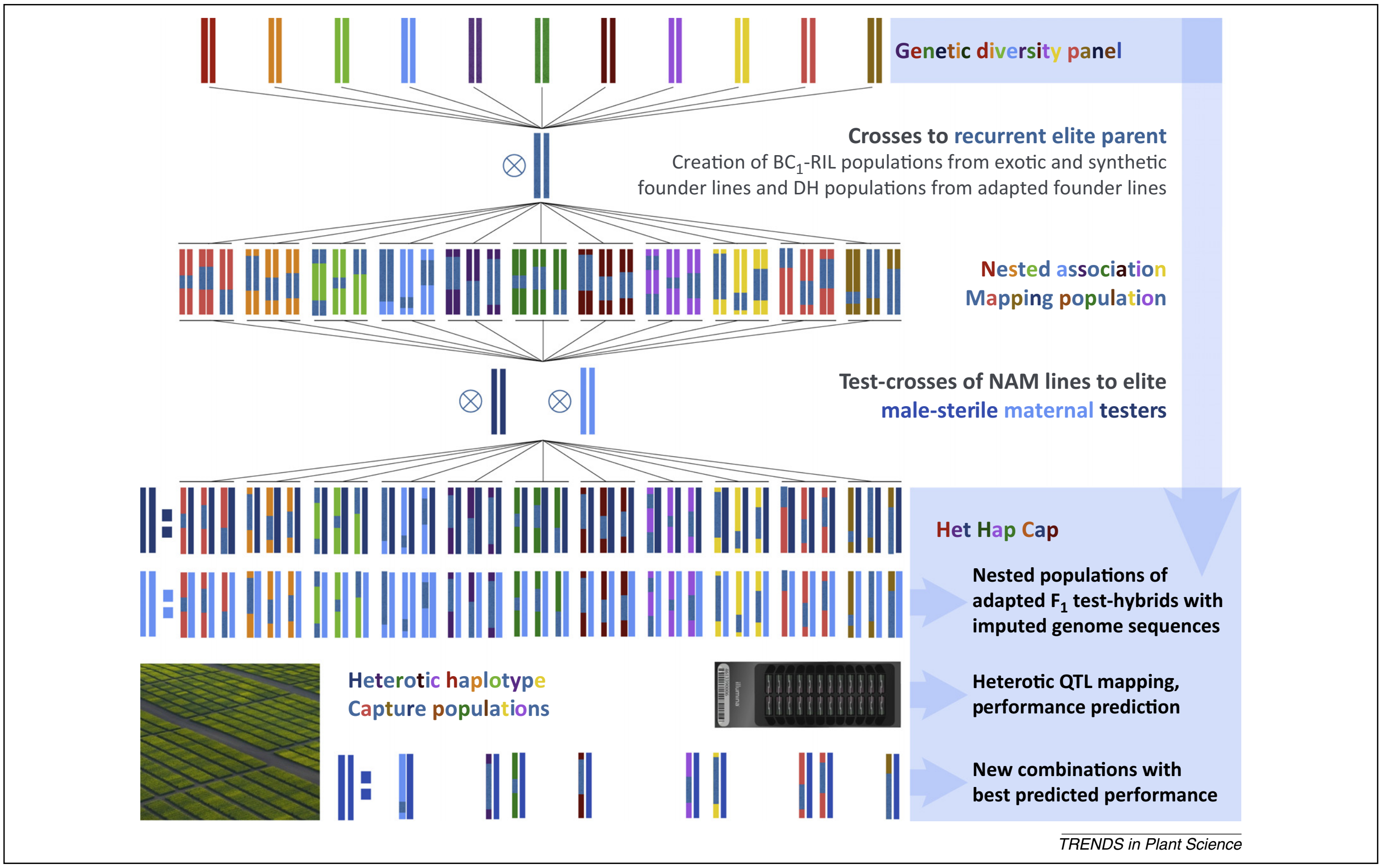
Cost-effective genome profiling technologies provide a powerful toolbox for the prediction of crop performance and introgression of novel diversity. However, even with the recent availability of genome sequences and high-density genotyping platforms for complex crop genomes (reviewed in [3]), genetic gain cannot improve beyond the diversity in available adapted breeding materials. To overcome this problem we developed a strategy to systematically capture and recombine novel genetic diversity, spanning the primary and secondary gene pools of *B. napus*, in an elite genetic background. The *B. napus* HHC populations (Figure 1) comprise an extensive panel of F_1 test hybrids

Glossary

Allopolyploidisation: an evolutionary process involving the formation of a fertile hybrid between two different but related species. The allopolyploid carries the genomes of both progenitor species.
Epistatic interaction: the term epistasis refers to genetic interactions where the expression of a gene is influenced by the expression of another, independently inherited gene.
Haplotype: a set of DNA variants, or polymorphisms, that tend to be inherited together, often because they are very close together in the same chromosome region and therefore less likely to be separated by crossing over.
Heterosis: the improvement in performance that is often seen in offspring from crosses between two different, inbred parents.
Homoeologous chromosome exchanges: in a diploid organism, each chromosome normally pairs with its partner during meiosis. This is known as normal, homologous chromosome pairing. In an allopolyploid (see above), chromosomes sometimes pair not only with their homologue but also with similar chromosomes derived from a different parental species. This is known as homoeologous pairing.
Hybrid varieties: heterosis (see above) is exploited in plant breeding by producing pure hybrid seed from controlled crosses between two genetically stable inbred parents. Hybrid varieties tend to have higher yield and more stable performance.
Insertion–deletion mutation (InDel): a given DNA sequence may be present in one individual but absent in the other. If we do not know whether the ancestral form of the sequence changed because of an insertion (leading to the presence of a new sequence) or a deletion (leading to the absence of the sequence) we describe this kind of mutation as an InDel.
Linkage drag: describes the phenomenon by which selection of a mutation with a desirable effect can lead to co-selection of neighbouring genes in the same chromosome region that have a negative effect.
Male-sterility system: because the flowers of most crops have both male and female organs, production of hybrid crop varieties (see above) requires a way to prevent self-pollination to ensure that pure hybrid seed is produced by a mother plant. To do this breeders use male-sterile mutants that cannot self-pollinate because they do not produce pollen.
Predictive breeding: a new method of breeding in which performance for a given trait in an untested hybrid is predicted based on the genome profiles of its parents.

Corresponding authors: Abbadi, A. (a.abbadi@npz-innovation.de); Leckband, G. (g.leckband@german-seed-alliance.de).
Keywords: genetic diversity; plant breeding; genome sequencing; heterosis; genomic prediction.

1360-1385/
http://dx.doi.org/10.1016/j.tplants.2015.04.013



TRENDS in Plant Science

Figure 1. Heterotic Haplotype Capture (HHC) for identification of heterotic diversity. HHC recombines novel genetic diversity in populations of elite F_1 test hybrids within an adapted genetic background. Nested association mapping (NAM) populations, whose members' genome sequences are imputed from parental genomes and high-density SNP data from the NAM lines, are crossed with fully sequenced male-sterile testers. Performance data from the HHC populations, in combination with their heterozygous genome profiles, provides exhaustive information for quantitative genetic analyses of heterosis, simultaneously enabling development of models for genomic prediction of hybrid performance. A male-sterility system for which all known *Brassica napus* accessions are restorers enables any NAM line to be combined with a male-sterile mother for which a maximum hybrid performance is predicted. Using the prediction models, the whole-genome profile of a new maternal line can be used to select the most promising new hybrid combinations for comprehensive field testing.

generated by controlled pollinations of elite male-sterile maternal parents with members of a large, interrelated genetic diversity panel. This recombination panel comprises more than 2500 half-sibling doubled-haploid (DH) or recombinant inbred line (RIL) progenies, generated using a nested association mapping (NAM) population design [4,5] from 50 divergent founder accessions, each crossed with a common elite parent.

Mixing up the gene pool by *de novo* allopolyploidisation

Thirty of the founder accessions were selected to sample allelic variation as evenly as possible across the gene pools of diverse winter-type *B. napus* morphotypes, including fodder rapes, kales, and old European and Asian oilseed forms (*B. napus* ssp. *napus*), along with rutabagas (*B. napus* ssp. *napobrassica*). The remaining 20 founder accessions comprise synthetic *B. napus*, generated through interspecific hybrids of genetically highly divergent representatives of the *Brassica* A and C diploid donor genomes of the allopolyploid *B. napus*. The use of synthetic *B. napus* derived from *de novo* interspecific hybridisation goes considerably beyond the few interspecific hybridisations from which this species is thought to have originated [6], thus introducing novel diversity into the breeding pool. Furthermore, *de novo* synthesis of synthetic *B. napus* increases recombination [7] and introduces novel presence–absence variation and gene conversions derived from extensive homoeologous chromosome exchanges [1]. Both phenomena are expected to elevate heterotic potential [8]. For example, complementary gene expression patterns corresponding to insertion–deletion polymorphisms (InDels) in different hybrid pools have been demonstrated to contribute to heterosis in rice (*Oryza sativa*) [9].

For production of the HHC populations, hybrid F_1 seed was produced in pollination tents using the commercial male-sterility system Male Sterility Lembke (MSL) (NPZ Lembke, Hohenlieth, Germany), for which all known *B. napus* pollinators restore fertility. This system allows test hybrids to be generated from large populations of pollinators, avoiding the time-consuming integration of fertility restoration loci that is generally needed for hybrid seed production.

Imputed genome sequences

The panel of 51 parental lines and one MSL tester were subjected to genomic resequencing with ~12–15× genome coverage on the Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA). From a total of around 4.7 billion