Plant Genetics in the Era of Modern Genomics

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International genome research efforts of various model and crop species have produced an unprecedented amount of information on genetic organization and variation. Such consortia united multidisciplinary research teams from all over the world, each of them contributing with their own expertise, infrastructure and facility services. The completion of such projects not only shed light on fundamental aspect of genome biology, but also revealed a plethora of genetic information and genome characteristics for medical research, as well as plant and animal breeding. The enormous advances of genomics were to a great extent made possible by next-generation sequencing technologies, but also benefitted from novel and powerful bioinformatics tools, statistics, comparative biosystematics and chromosome biology. In addition, large comparative genome projects, like the 1000 human (http://www.1000genomes.org/) and 1000 *Arabidopsis* genomes initiatives (http://www.1001genomes.org/), have revealed a treasure of most common single nucleotide polymorphisms (SNPs) and InDels, and many larger structural rearrangements between human persons, genotypes and related wild species.

In my presentation I will show some examples of recent studies that combine genetics, bioinformatics, genomics and chromosome research in a couple of plant model and crop species, and include analyses of tomato, potato and *Arabidopsis thaliana* genomes (Szinay *et al*., 2008; Szinay *et al*., 2012; Tang *et al*., 2008; tomato genome consortium, 2012; Peters *et al*., 2012). The aims of these analyses were to confirm chromosomal positions for genetic markers and contigs on the cell complements using Fluorescent *in situ* Hybridization of BACs, plasmids and PCR fragments as probes on mitotic and pachytene cells, and naked DNA fibers (Fransz *et al*., 1996; de Jong *et al*., 1999). Our study demonstrated positions of annotated contigs and the size of the gaps in between them, and so supported directly the bioinformatics and de novo assembly of the pseudo-molecules. In addition, we were able to elucidate chromosome rearrangements, repeat dynamics, centromere and heterochromatin locations and were able to interpret linkage drag and other sources of problems in breeding programs. The existence of several inversions and translocations were demonstrated between tomato and potato, and several other species of the Solanaceae family, and helped to further support phylogenetic studies in the tomato and potato clades.

Of special interest was the elaborative study on a 1.17 MB paracentric inversion that we observed in the *Arabidopsis* accession Columbia. Chromosome painting experiments with probed BACs and PCR products on pachytene chromosomes, extended DNA fibers and interphase nuclei of Columbia, Landberg *erecta* and related accessions, with and without the inversion, narrowed down the breakpoint regions at an accuracy of less than 1 kb. Further comparison of the DNA sequences of both breakpoints revealed nucleotide precision of the inversion. The rearrangement that occurred about 10,000 years ago was most likely induced by a *vandal* transposon involving transfer of the pericentromere heterochromatin region with a F-box protein-coding gene into the short arm euchromatin of chromosome 4. Epigenetic analyses showed that the breakpoints demarcate sharp transitions of DNA methylation at the heterochromatin – euchromatin borders hinting at a recent history of the inversion. Microscopic observations of the chromosome pairing and genetic analysis of the Col X Ler hybrid revealed normal pairing but total lack of crossovers in the region heterozygous for the inversion. AFLP analysis revealed a conspicuous sequence similarity in the inversion region between the inversion containing Col-0 and Ws-2, com-pared to sequences outside the inversion. An additional large-scale SNP analysis of 1200 ac-cessions demonstrated extreme low levels of polymorphisms in the inversion region of 48 accessions from Europe and the USA. Such a distribution, which may have occurred less than few hundred years ago, suggests that the inversion spread across large areas of the world through introgressive hybridization. Ancestral crossover site just outside the inversion near the distal and proximal breakpoints were extremely rare and so could be used as landmarks in recent distribution of populations carrying the inversion bearing genotype to other regions in the world.

We also made use of two genetic tools recently developed for *Arabidopsis*, the *quartet*1 mutation (Copenhave *et al*., 2000), which keeps the four daughter cells of a single male meiosis together, and so allowing single meiosis analyses, and the tail swap CenH3 construct for haploid production (Ravi *et al*., 2010). Combining these tools makes it possible to generate four haploid (or dihaploid) plants that came from one and the same meiosis and so enables fine mapping of crossover sites and gene conversions at SNP level accuracy. Besides the ten crossovers in most male meioses, most DSBs are 'simply' repaired as non-crossovers. Following the SNPs on the chromosomal DNA of the four plants from a single meiosis event now reveals precise detection of crossovers and gene conversions. In *Arabidopsis*, however, we have found that gene conversions are extremely rare, likely due to the very short gene-conversion tracts. This observation stands in strong contrast to recent related reports claiming hundreds of gene-conversions in single Arabidopsis and yeast meioses.

Another milestone of applied chromosome biology in plant genetics is the reverse breeding technology that we described in detail recently (Wijnker *et al*., 2008; Dirks *et al*., 2009; Wijnker *et al*., 2012). This strategy demonstrates that full suppression of crossovers in heterozygous Arabidopsis meiosis leads to low frequencies of non-recombinant chromosomes offspring. To this end plants generated as doubled haploids consist of all kind of non-recombinant parental chromosome combinations. After genotyping, one can select 'complementing' sets of doubled haploid offspring that – when crossed – give rise to hybrids that are identical to the mother plant (which is the starting heterozygote). Reverse breeding can also be used to generate chromosome substitution lines. When two accessions are crossed (*i.e.*, we used the natural accessions Columbia and Landsberg *erecta*) to generate an F1, the reverse breeding offspring of these will consist of all possible chromosome combinations. We are currently completing so called Chromosome Substitution Libraries (CSLs) in which all 32 possible chromosome combinations are represented. Such a collection of lines encompassing the smallest possible RIL population for the species, and can be used in a whole variety of breeding experiments. We are currently exploring the use of these lines for the dissection of heterosis in *Arabidopsis*.

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