Spotlight

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A new chromosome was born: comparative chromosome painting in *Boechera*

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Comparative chromosome painting is a powerful tool to study the evolution of chromosomes and genomes. Analyzing karyotype evolution in cruciferous plants highlights the origin of aberrant chromosomes in apomictic *Boechera* and further establishes the cruciferous plants as important model system for our understanding of plant chromosome and genome evolution.

Comparative chromosome painting: a powerful tool to bridge cytogenetics, genomics and evolutionary biology

Chromosome painting was introduced more than 25 years ago [1] as method to visualize chromosomes or larger chromosome fragments using fluorescent-labeled chromosome-specific homologous DNA probes. However, repetitive sequences in the target as well as the probe DNA often resulted in non-specific binding and hybridization signals. Therefore extensive blocking with excess of unlabeled total genomic DNA was required, ideally enriched for repetitive fraction, and the term chromosomal *in situ* suppression was introduced as synonym for chromosome painting [2]. Comparative chromosome painting (CCP) uses cross-specific hybridization of fluorescent-labeled probes of different size. Given a defined reference genome as source for these probes (e.g. *Arabidopsis thaliana*) and assuming chromosome collinearity among the species to be compared, CCP provides a powerful tool box to reconstruct chromosome and genome structure, as well as the evolutionary history of chromosomes, karyotypes, and whole genomes [3]. Despite more than 30 million years of Brassicaceae crown group evolutionary history the Brassicaceae genomes show very high levels of collinearity among all evolutionary lineages, which allows for the reliable cross-species identification of large chromosome homologues via CCP using *Arabidopsis thaliana* chromosome-specific BAC (Bacterial Artificial Chromosome) contigs representing 24 genomic blocks of the entire ACK (Ancestral Crucifer Karyotype).

Boechera: not only a model system for the evolution of apomictic reproduction

The genus *Boechera* (Böcher's rock cress) from the crucifer family is one of the few model systems available to study the evolution of apomixis in plants, the production of seeds without sex [4,5]. This genus has a Pleistocene evolutionary history going back 2.5 million years and radiated within the last two million years into several main evolutionary lineages. Currently more than 110 species are found all over the North American continent [6].

Boechera evolution was highly affected by extensive hybridization, introgression and reticulate evolution (Figure 1A–C). Nearly 50% of the members of the genus *Boechera* are polyploid (triploid) hence contributing substantially to overall species diversity. These polyploids are normally characterized by apomictic reproduction. However, within the genus *Boechera* we do find the exceptional case of diploid apomictic species with 2n = 14 chromosomes, leading to the question if the genetic basis of apomixis can be unraveled through analyzing these diploid species. Moreover, aneuploid diploid cytotypes with 2n = 15 were described in detail, their apomictic nature was shown [7] and their continent-wide distribution (in North America) has been illustrated [8], but it was unclear if these heterochromatic B-like chromosomes were actually carrying genetic elements associated with the apomictic phenotype.

Using CCP the origin and evolution of aberrant chromosomes was studied in detail [9]. This study built upon past results of the same research group on various Brassicaceae species (Martin Lysak and co-workers, Brno) and other work describing Ancestral Crucifer Karyotype (ACK) with n = 8 chromosomes [10]. The key results of Lysak's team highlight close phylogenomic relationships between the n = 8 ACK and the n = 7 *Boechera* genomes; whereby only four major chromosome rearrangements are needed to explain the evolution from ACK to *Boechera* genome. Notably, one ancestral chromosome (AK5) completely fused with two other chromosome fragments, and the position of its original centromere is identifiable and may be the focus of future studies focusing on its inactivation and subsequent deletion.

The biologically most fascinating aspect of the work introduced by Mandáková and co-workers [9] is the evolution of a new chromosome (*Heť*) from largely heterochromatic *Het* chromosome, derived from *Boechera* chromosome 1 (BS1). This BS1 chromosome was formed via reciprocal translocation between AK1 and AK2 also constituting a new 'hybrid centromere' being the respective starting point for heterochromatin accumulation (Figure 1 E, F). The *Het* chromosome underwent further mutational steps including a centric fission and pericentric inversion creating another smaller *Heť* version

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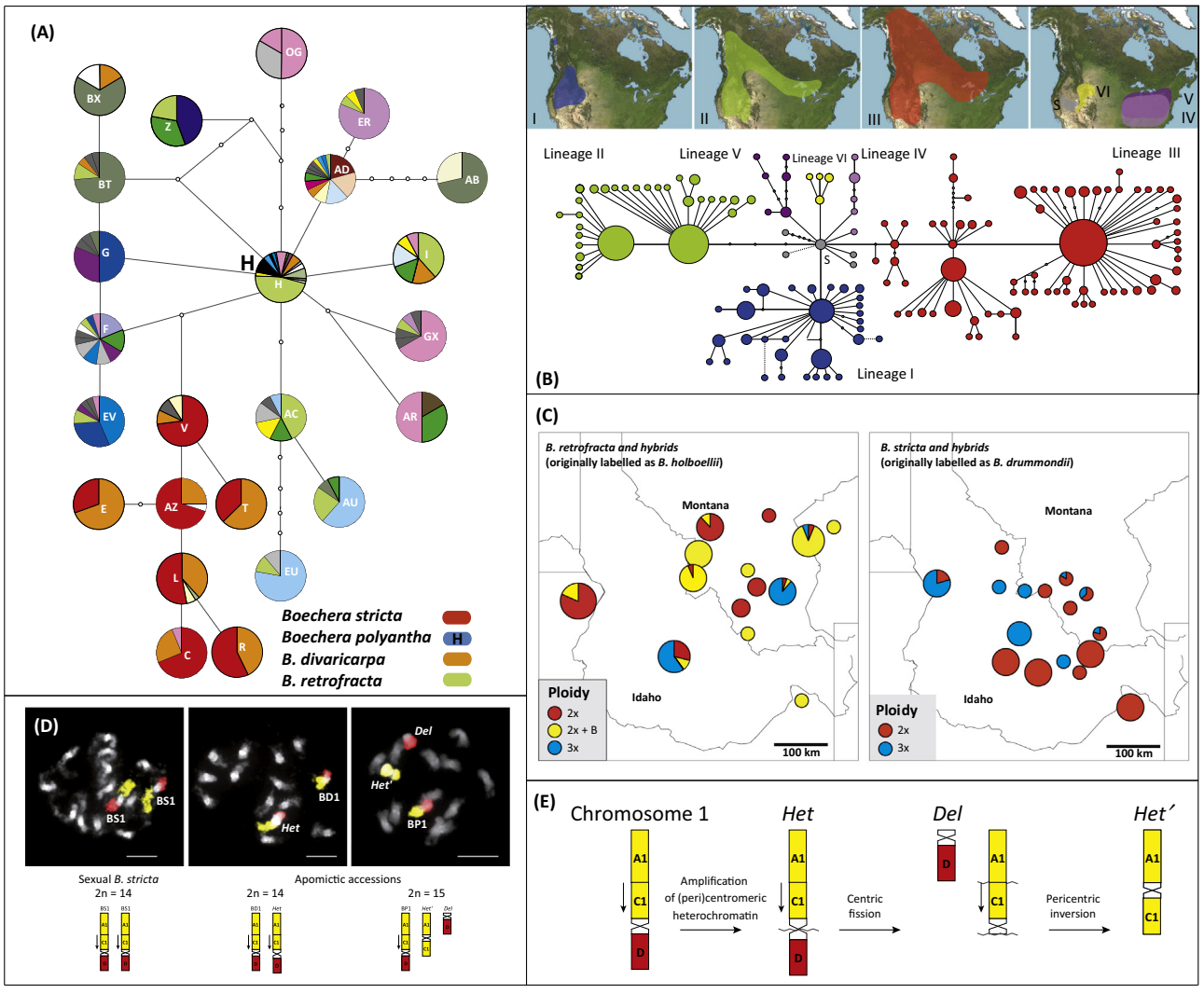

TRENDS in Plant Science

Figure 1. From Genepools to genomes. **(A)** Illustration of the extensive sharing of ITS sequence types (internal transcribed spacer of nuclear encoded ribosomal DNA) among the different *Boechera* species that are represented by different colors (redrawn from [12]), taxa focused on in [9] are highlighted in the bottom right corner **(B)** Evolutionary lineages of the genus *Boechera* demonstrated by a chloroplast DNA haplotype network (redrawn from [5]). Several evolutionary lineages have a restricted distribution range and highlight potential refuge areas (e.g. lineage VI and V/VI). Other major lineages (I, II, III) have significantly overlapping distribution ranges. (A) and (B) indicate extensive reticulate evolution (hybridization and introgression) all over the North American continent and consequently do contain numerous triploid apomicts. **(C)** Geographic distribution of an extra B chromosome exemplified for northwestern North America (redrawn from [8]. **(D)** Identification of BS1 and aberrant chromosomes (*Het, Het́, Del*) using CCP at mitotic metaphases stages (taken from [9]) and **(E)** hypothesized origin of the aberrant chromosomes from a BS1 homologue (taken from [9]).

plus a small fragment called *Del* chromosome explaining the cytogenetic situation in apomictic 2n = 15 species. The newly formed chromosomes (*Het́* and *Del*) are persisting in apomictic species and thus can survive in secure "genetic havens". However, because of frequent reticulate evolution and hybridization (Figure 1) this chromosome (*Het́*) can easily move into other genomes and again persists if apomixis is the prevailing mode of reproduction. It will be intriguing to see if there is a potential causal link between occurrence of these aberrant 'apomictic' chromosomes and the apomixis trait. Some first candidate (apo)alleles (APOLLO and UPGRADE), involved in gametophyte and embryo development, have been characterized [11], but direct experimental evidence for their position on *Het* is still lacking. However, their continental-wide occurrence in the various genepools does fit perfectly with the idea of the evolution of apomixis in the genus *Boechera* [9].

The exciting results by Mandáková *et al*. [9] highlight the interplay of cytogenetic analyses, genomics, and evolutionary biology and contribute significantly to our overall understanding of evolution of apomixis in *Boechera*. CCP is a powerful technique to unravel the history of genomes and its chromosomes, and, therefore, will play an increasingly important role in evolutionary biology. It will also substantially contribute, at least within Brassicaceae family, in generating high quality genome assemblies of many species.

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