Research review

Live and let die: centromere loss during evolution of plant chromosomes

Author for correspondence:
Martin A. Lysak
Tel: +420 549 494 154
Email: martin.lysak@ceitec.muni.cz

Received: *19 February 2014*
Accepted: *11 May 2014*

Martin A. Lysak

Plant Cytogenomics Research Group, CEITEC – Central European Institute of Technology, Masaryk University, CZ-625 00 Brno, Czech Republic

New Phytologist (2014) **203:** 1082–1089
doi: 10.1111/nph.12885

Key words: centromere inactivation, centromere repositioning, dicentric chromosomes, dysploidy, karyotype evolution, nested chromosome insertion, terminal chromosome translocation.

Summary

Functional centromeres, ensuring regular chromosome segregation in mitosis and meiosis, are a prerequisite for the evolutionary success of pre-existing and new chromosome variants. The rapid progress in plant comparative genomics and cytogenetics brings new insights into the evolutionary fate of centromeres and mechanisms of chromosome number reduction (descending dysploidy). Centromere loss and relocation in chromosome regions with otherwise conserved collinearity can be explained by conventional mechanisms of chromosome rearrangements or, as newly available phylogenomic and cytogenomic data suggest, by centromere inactivation through epigenetic chromatin modifications and/or intra- and inter-chromosomal recombination.

Introduction

Chromosome numbers of plants vary enormously over a 360-fold range (from $n = 2$ in five angiosperm species to $n = 720$ in the fern *Ophioglossum reticulatum*; Cremonini, 2005; Khandelwal, 2008). Besides the increase in chromosome number as a result of whole-genome duplication (polyploidy) and increases/decreases through aneuploidy, chromosome numbers are altered by chromosome rearrangements towards higher and lower values (ascending and descending dysploidy, respectively). Owing to the fast progress in comparative genomics and cytogenetics in families harboring major crop and model species, such as grasses (Poaceae: The International Brachypodium Initiative, 2010; Luo *et al.*, 2009, 2013; Wang & Bennetzen, 2012), crucifers (Brassicaceae: Lysak *et al.*, 2006; Mandáková *et al.*, 2010a,b; Cheng *et al.*, 2013), Cucurbitaceae (Huang *et al.*, 2009; Yang *et al.*, 2014), Solanaceae (Sierro *et al.*, 2013; The Tomato Genome Consortium, 2013), and Rosaceae (Jung *et al.*, 2012), a new light was cast on the mechanisms of chromosome number reduction in plants. Although scant phylogenomic data do not allow us to perform a rigorous statistical analysis of the incidence of descending dysploidy, we know that the process is frequent, occurs in different phylogenetic lineages, and almost inevitably follows whole-genome duplications during so-called genome diploidization (The Arabidopsis Genome Initiative, 2000; Mandáková *et al.*, 2010a, b; Murat *et al.*, 2010; Salse, 2012; Wang & Bennetzen, 2012; Cheng *et al.*, 2013). The opposite process (i.e. chromosome number increase by chromosome fissions) seems to be less common, taking into account genome comparisons of analyzed angiosperm genera and families (but see Fishman *et al.*, 2014). Karyotypes with telocentric chromosomes resulting from chromosome fissions have so far only been reported in a few groups, for example, in faba bean (Schubert *et al.*, 1995), orchids (Cox *et al.*, 1998), and the spiderwort family (Comelinaceae: Jones, 1998).

For both ascending and descending dysploidy in plants with monocentric chromosomes, 'a change in the number of chromosomes means a change in the number of centromeres' (Darlington, 1937). Presumably inactivated or lost centromeres are identified through comparisons of genomes of extant species, on the one hand, and between extant and reconstructed ancestral genomes on the other. Recently, several instances of centromere loss and relocation in chromosome regions with otherwise conserved collinearity were reported (Y. Han *et al.*, 2009; Luo *et al.*, 2009, 2013; Mandáková *et al.*, 2010a,b; Hu *et al.*, 2011; Cheng *et al.*, 2013). Interestingly, some of these centromere losses are not explainable by conventional descending dysploidy (i.e. by unequal reciprocal translocation and loss of the small product) and require other interpretations.

Here I summarize the recent progress on understanding mechanisms of descending dysploidy associated with centromere elimination in the light of newly available phylogenomic and cytogenomic data for several angiosperm families.

Plant centromeres

As the structure and evolution of plant centromeres have been covered in a number of insightful reviews (Malik & Henikoff, 2002, 2009; Ma *et al.*, 2007a; Birchler *et al.*, 2011; Birchler & Han, 2013a), only a brief account of the plant centromere properties is given here. The role of centromeres is to mediate sister chromatid cohesion up to a faithful chromatid segregation through kinetochore interaction with spindle microtubules during mitotic or second meiotic division. Typical plant centromeres comprise arrays of centromere-specific satellite repeats and retrotransposons (particularly long terminal repeat (LTR) retrotransposons). Centromeric chromatin is characterized by centromere-specific repeats and by substitution of histone H3 with the centromere-specific histone H3 variant (CenH3) within the core of centromeric nucleosomes (Howman *et al.*, 2000; Blower & Karpen, 2001). Often centromeres are flanked by repeat-rich pericentromeric heterochromatin. Whereas the centromere function and kinetochore proteins are evolutionarily conserved across eukaryotes (Henikoff *et al.*, 2001; Birchler *et al.*, 2011), the centromere size, amino acid sequence of CenH3, and the sequence and copy number of centromeric repeats are not, and could vary among (sub)species, populations, and individuals and even between centromeres within a chromosome complement. This variation is particularly typical for rapidly evolving centromeric satellites and proteins (i.e. CenH3 and/or CENP-C) (Ma & Bennetzen, 2006; Ma *et al.*, 2007a; Gong *et al.*, 2012; Wang *et al.*, 2014). The puzzling rapid evolution of centromeric repeats and CenH3-encoding proteins has been explained by the centromere-drive model (Henikoff *et al.*, 2001; Malik & Henikoff, 2002, 2009). According to this model, expansion of centromeric repeats results in larger CenH3 domains with increased microtubule-binding capacity during asymmetric first nuclear division in female meiosis. The resulting preferential transmission of the corresponding chromosomes to the egg will increase the proportion of the larger centromeres in a population. To counteract detrimental effects of this drive, CenH3 alleles with mutated DNA-binding specificity would evolve as suppressors. These counteracting processes could explain the rapid divergence of centromeric repeats and CenH3.

Collectively, these findings changed the traditional perception of centromeres as static structures on the chromosome (Birchler *et al.*, 2011). It seems that centromeres are not exempted from a tug of war between genome expansion (mainly as a result of retrotransposon amplification) and genome downsizing. The proliferation of transposable elements is counterbalanced by removal of DNA repeats by unequal homologous recombination (UR) and illegitimate recombination (IR) (Bennetzen *et al.*, 2005; Vitte & Bennetzen, 2006; Hawkins *et al.*, 2009). Both UR and IR were identified as major forces mediating the removal of LTR retrotransposons from plant centromeres (Ma & Bennetzen, 2006; Ma *et al.*, 2007a). UR and IR can also be involved in removal of repetitive components of nonfunctional (inactive) centromeres. By contrast, it is assumed that the 'maturation' of newly formed repeat-less centromeres is associated with subsequent *de novo* amplification and insertion of satellite repeats into CenH3 domains (Gong *et al.*, 2012; Wang *et al.*, 2014), and reinforced by centromere drive. Centromere-specific repeat arrays can further expand through segmental duplications and proliferation of (LTR) retrotransposons (Ma & Bennetzen, 2006; Ma & Jackson, 2006; Ma *et al.*, 2007a; Wu *et al.*, 2009).

Translocation-induced centromere loss

Descending dysploidy can occur through a translocation event involving terminal regions of two chromosomes – usually with breakpoints close to the centromere of the long arm of a telo- or an acrocentric chromosome and one end of any type of chromosome. Such events, sometimes called end-to-end fusions or telomeric chromosome fusions (Salse, 2012), result in a large chromosome comprising most parts of the original chromosomes and a small centromere-containing minichromosome. These terminal chromosome translocations (TCTs), that is, translocations with terminal breakpoints, are mediated by nonhomologous end joining (NHEJ) or by homologous recombination at DNA double-strand breaks (DSBs) within (sub)telomeric tandem repeats, rDNA repeats of terminal nucleolus organizing regions (NORs) or (peri)centromeres. TCTs include so-called Robertsonian translocations, that is, whole-arm translocations (sometimes confusingly called centric fusions), that transform two telocentric or acrocentric (rod-shaped) chromosomes into one V-shaped (sub)metacentric chromosome (Darlington, 1937; Tobgy, 1943; Jackson, 1971; Lysak *et al.*, 2006; Schubert & Lysak, 2011; Stimpson *et al.*, 2012). The minichromosome product of a TCT is supposed to contain a centromere, repeats and other dispensable sequences, and to be meiotically unstable because of an inability to form chiasmata and segregate regularly and/or because of insufficient sister chromatid cohesion during the first meiotic division (Birchler & Han, 2013b). It is conceivable, though, that such a centric fragment with telomeres at both ends becomes fixed in a chromosome complement as a B chromosome by accumulation of sequences from the remaining autosomes, and from mitochondrial and/or plastid genomes (Martis *et al.*, 2012).

A centromere can also be eliminated as a result of a misrepair of multiple DSBs in two nonhomologous chromosomes yielding diverse products, for example, a monocentric chromosome with a zebra-like pattern of chromosome segments largely collinear to the corresponding regions of the participating chromosomes. The fragment containing the second centromere is meiotically unstable and sooner or later becomes eliminated. This process of chromosome rearrangement mediating a reduction in chromosome number and centromere loss was described by Zhang *et al.* (2008) in an alloplasmic wheat line and might be more common, in particular, in allopolyploid genomes undergoing diploidization through descending dysploidy (e.g. Mandáková *et al.*, 2010a).

Centromere loss through 'nested chromosome insertion'

Nested chromosome insertion (NCI) is a mechanism of descending dysploidy described for grass species by Luo *et al.* (2009). NCI is a translocation event involving two nonhomeologous chromosomes, whereby the entire donor chromosome appears to be 'inserted' into or near the centromere of the recipient chromosome (Luo *et al.*, 2009; Murat *et al.*, 2010). This translocation event requires at least three breakpoints: two at telomeres of the inserted chromosome and one at the (peri)centromeric region of the recipient chromosome (Schubert & Lysak, 2011). NCIs occurred independently in different subfamilies and tribes of the grass family and contributed to chromosome number reduction from the ancestral 12 chromosomes to 10 (sorghum, Luo *et al.*, 2009), seven (Triticeae: *Aegilops tauschii*; Luo *et al.*, 2009, 2013) or five chromosomes (*Brachypodium distachyon*, The International Brachypodium Initiative, 2010). NCIs presumably also contributed to chromosome number reduction from $n = 20$ to $n = 10$ in maize (Wang & Bennetzen, 2012). The insertion may target the core centromere (location of the kinetochore) or regions close to the centromere; both instances were reported (Luo *et al.*, 2009, 2013; Wang & Bennetzen, 2012). If the recipient centromere is not disrupted, the resulting chromosome becomes dicentric. Although the fate of the centromere of the recipient chromosome is elusive, it seems reasonable to assume that the centromere, if not deleted in the course of DSB repair, becomes inactive by epigenetic modifications (see later). An inserted chromosome could leave its signature in the form of interstitial (sub)telomeric repeats within the composite chromosome. However, no remnants of telomeric repeats were detected in the insertion junctions in *Brachypodium* (T. Gu & W. Belknap, pers. comm.; Murat *et al.*, 2010).

Nested chromosome insertion appears to be the predominating mechanism of descending dysploidy in grasses (Murat *et al.*, 2010), although other types of chromosome rearrangements reducing chromosome numbers can also occur in Poaceae (Wang & Bennetzen, 2012). We do not understand why NCIs are prevalent in grasses. NCI outside Poaceae was claimed to play a role in the origin of chromosome C1 in cucumber (Yang *et al.*, 2014) and of three composite chromosomes in three genera of Brassicaceae. In *Hornungia alpina*, chromosome AK2 was integrated into AK5 (Lysak *et al.*, 2006), and the ancestral chromosome PC3 was formed by insertion of AK2 into the centromere of AK3 in the genus *Pachycladon* (Mandáková *et al.*, 2010b). An NCI event can be assumed for the origin of the complex chromosome AK5/8/6 in the hypotetraploid *Cardamine pratensis* by insertion of chromosome AK5 into the centromere of translocation chromosome AK8/6 (Mandáková *et al.*, 2013). However, NCIs cannot be objectively assessed in groups of organisms for which advanced comparative genomic and cytogenomic data are lacking.

Dicentric chromosomes

Whereas TCTs resulting in monocentric chromosomes and the elimination of one centromere through loss of the small translocation chromosome were deduced in the early days of plant cytogenetics (Darlington, 1937 and references therein; Tobgy, 1943), the origin and stable maintenance of dicentric chromosomes as a result of asymmetric translocations were not fully acknowledged until recently. Following McClintock's pioneering research on maize, dicentrics entering the breakage–fusion–bridge (BFB) cycle (McClintock, 1939) were considered as unstable and lacking a long-term evolutionary prospect. Dicentric chromosomes are formed by asymmetric reciprocal translocation usually between centric ends or short arms of two telocentrics or acrocentrics, respectively (although theoretically any two chromosomes can be involved). An acentric fragment, frequently too small to be detected microscopically, is formed as the second translocation product (Fig. 1). The fate of the dicentric chromosome and its two centromeres depends on the position of breakpoints, and thus on the distance between the two centromeres (Stimpson *et al.*, 2012). If these centromeres are in close proximity, both can remain active (Sears & Câmara, 1952; Schubert *et al.*, 1995). Similarly, when two different centromeres are broken and then brought together by recombination, both can stay active and constitute one functional 'hybrid centromere' (Zhang *et al.*, 2001). The maximum distance between two centromeres still ensuring a regular segregation was inferred as 20 Mb in engineered human dicentric chromosomes (Higgins *et al.*, 2005). However, the larger the distance between the centromeres, the higher is the probability that the dicentric

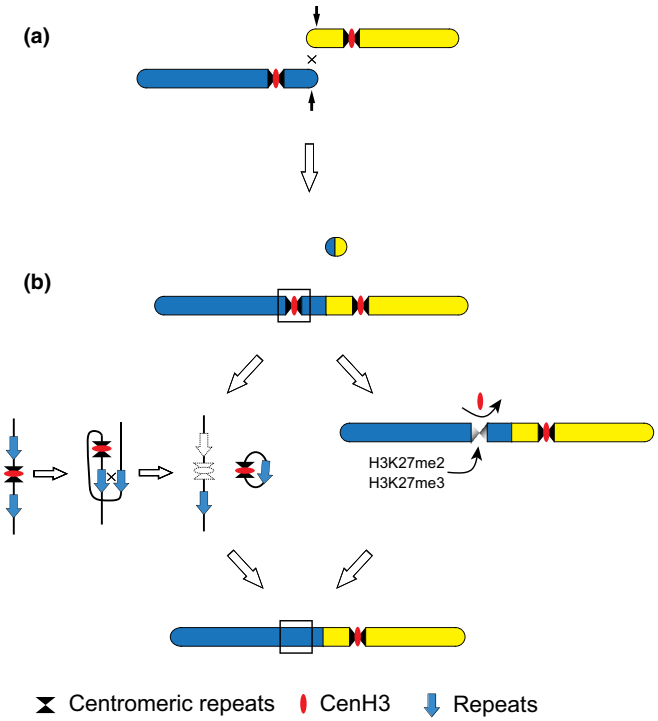

Fig. 1 Potential modes of centromere inactivation and loss. (a) Asymmetric reciprocal translocation between two nonhomologous chromosomes results in the origin of a dicentric chromosome and an acentric fragment. (b) The dicentric chromosome is stabilized by deletion of one centromere. This occurs either by recombinational sequence removal (schematically left) or by epigenetic inactivation retaining the original DNA sequences (right), or by partial loss of centromeric sequences in combination with epigenetic inactivation. See text for details. H3K27me2, H3 lysine 27 dimethylation; H3K27me3, H3 lysine 27 trimethylation.

chromosome will break in the region between the centromeres (BFB cycle; e.g. see Lukaszewski, 1995), because the risk of attachment of sister centromeres to fibers from the same spindle pole, as a result of twisting of sister chromatids between the centromeres, increases with the distance. If a dicentric chromosome with distantly positioned centromeres should segregate regularly and become fixed in a population, one of the two centromeres has to become inactive or deleted (Fig. 1).

Dicentric chromosomes and centromere inactivation in plants

As outlined earlier, dicentric chromosomes usually undergo BFB cycles (McClintock, 1939; Lukaszewski, 1995). A transmissible chromosome with two centromeres was observed for the first time in wheat (Sears & Câmara, 1952). New insight into the structure of dicentric chromosomes was gained with the advent of fluorescence *in situ* hybridization (FISH) of centromeric and telomeric repeats, and immunofluorescent detection of centromere-specific histone variants. Schubert *et al.* (1995) showed that recombination between centric ends of two telocentrics in the field bean may result in a meta(di)centric chromosome with telomeric repeats still persistent between the two centromeres. Breakage between the centromeres of the dicentric led to restitution of two telocentric chromosomes. This showed that both centromeres and telomeric repeats can temporarily form one functional centromere, and these components can again become functional in fission products. In wheat–rye translocation lines, Zhang *et al.* (2001) showed that centric breakage followed by misrepair frequently results in intergenomic translocation chromosomes with a wheat–rye 'hybrid' centromere, and that the chromosomes with composite centromeres were stable during mitosis and meiosis. Employing FISH and antibodies against the centromeric histone H3 variant CenH3, Zhang *et al.* (2010) could show that centromeres of the transmissible wheat dicentric of Sears & Câmara (1952) actually comprise one large (primary) and two smaller domains, all three being positive for CenH3. Small domains were often observed as a single unit, albeit still smaller than the primary centromere. As the dicentric chromosome is transmitted to 70% of the progenies, the three centromere domains often behave functionally as one centromere. It seems likely that this is a result of their physical proximity and/or the dominating pulling force of the primary centromere in meiotic anaphase I. Nevertheless, some tricentrics have undergone breakage and centromere inactivation. The inactivation was always associated with small centromere domains and elevated concentrations of histone H3 lysine 27 di- and trimethylation (H3K27me2 and H3K27me3). In rice, a whole-arm reciprocal translocation led to the origin of a translocation chromosome with a tripartite centromere comprising two centromere-specific CentO tandem arrays separated by a 5S rDNA region *c.* 400 kb long. As only the two CentO satellite regions were positive for CenH3, the regularly segregating translocation chromosome has two active centromere regions (Wang *et al.*, 2013). The existence of di- and tricentric chromosomes segregating as functionally monocentric chromosomes was recently corroborated by the finding of extremely long (70–107 Mb) centromeres with multiple CenH3-containing domains in chromosomes of the pea (Neumann *et al.*, 2012). The unexpected 'metapolycentric' structure of pea chromosomes suggests that experimentally obtained, transmissible dicentric chromosomes may exist in wild species.

Experimental engineering of maize chromosomes showed that centromere inactivation is not extremely rare and can play a role in karyotype evolution. Han *et al.* (2006) found maize A chromosomes with an additional centromere translocated from a B chromosome. Although the recombinant A-B chromosomes contained duplicated centromeric sequences, only the smaller B chromosome centromere was inactive (negative for CenH3 and H3 serine 10 phophorylation). Similarly, if differently sized B chromosome centromeres occurred on one chromosome, the smaller centromere was always inactive and negative for CENPC, CenH3 and H3S10ph marks (F. Han *et al.*, 2009). Interestingly, when the inactive centromere was separated from the active one by intra-chromosomal recombination, the inactive centromere became active again. In maize, centromere inactivation is not restricted only to centromeres originating from B chromosomes, but can also affect for many generations centromeres derived from A chromosomes. The maize translocation chromosome T1-5 possesses an active and an inactive centromere (Gao *et al.*, 2011). The inactive centromere contained centromere-specific repeats (CentC and CRM), but neither CENP-C nor H3S10ph.

In evolutionary terms, centromere inactivation is documented only for maize and for crucifer species. Wang & Bennetzen (2012) reconstructed the fate of the 20 ancestral maize (peri)centromeres in the process of chromosome number decrease from $n = 20$ towards $n = 10$ of the extant maize genome. Centromere inactivation or loss was anticipated to contribute to the origin of current maize chromosomes, often following TCTs and NCIs. Out of 10 lost (peri)centromeres, only one is still detectable as co-occurring remnants of maize centromeric-specific satellite (CentC) and retrotransposons (CRMs) within the modern chromosome 10. This result supports the thesis that sequence removal can be preceded by functional (epigenetic) inactivation. A growing number of centromere inactivation or loss events has been reported for descending dysploidies in crucifer species. Sequence comparison between genomes of *Arabidopsis lyrata* ($n = 8$) and *Arabidopsis thaliana* ($n = 5$) revealed two remnants of ancestral centromeric satellite repeats on chromosome At2, at the position of the ancestral Al4 (AK4) centromere (Hu *et al.*, 2011). These data suggest that the *A. thaliana* chromosome At2 originated by a TCT event between short arms of ancestral chromosomes AK3 and AK4. The proposed alternative scenario (Lysak *et al.*, 2006), assuming a paracentric and subsequently a pericentric inversion of the short arm of AK4, followed by a translocation between the centric end of AK4 and the short arm of AK3, and resulting in the loss of the AK4 centromere as a dispensable centric fragment, is plausible but requires one step more than a TCT accompanied by inactivation of the AK4 centromere. Chromosome number reduction from $n = 8$ to $n = 7$ in *Boechera stricta* was also most probably associated with inactivation of the ancestral AK5 centromere on chromosome Bs5 (Schranz *et al.*, 2007). Interestingly, centromere inactivation is frequently observed in crucifer genomes that have descended from tetraploid or hexaploid progenitors. During diploidization of a

hexaploid ancestor ($n = 21$) towards *Brassica rapa* ($n = 10$), at least two out of 11 lost ancestral centromeres were lost without a break of collinearity (Cheng *et al.*, 2013). Whereas no sequence remnants of the ancestral centromere were left on the *B. rapa* chromosome A03, remnants of a pericentromeric retrotransposon and tandem repeats were detected at the site of the ancestral centromere on chromosome A04. Crucifer genera endemic to Australia and New Zealand have descended from one or two very similar ancestral tetraploid genomes ($n = 16$). In these taxa, centromere inactivation was apparently a prominent mechanism during descending dysploidy and the number of inactive ancestral centromeres is positively correlated with the extent of genome diploidization and chromosome number reduction. In the younger genus *Pachycladon* ($n = 10$), four ancestral centromeres became inactive (Mandáková *et al.*, 2010b). By contrast, five, six, and seven ancestral centromeres were found inactive in *Ballantinia antipoda* ($n = 6$), *Stenopetalum lineare* ($n = 5$), and *Stenopetalum nutans* ($n = 4$) (Mandáková *et al.*, 2010a).

Mechanisms of centromere inactivation and loss

As the molecular mechanism(s) of centromere inactivation remain elusive and the topic was covered by several recent reviews (Fu *et al.*, 2012; Kalitsis & Choo, 2012; Sato *et al.*, 2012; Stimpson *et al.*, 2012), we are only highlighting the main principles of how dicentric chromosomes may become stabilized by centromere inactivation and deletion. We distinguish between centromere inactivation and loss, although loss might follow inactivation, or both processes might concur (Fig. 1). Inactivation would include predominantly epigenetic modification of centromere-specific proteins, whereas centromere loss (deletion) is thought to be associated with a removal of centromere-specific sequences, for example, via misrepair of DSBs on opposite sites of a centromere, unequal sister chromatid exchange or by unequal recombination between LTR retrotransposons or centromeric tandem repeats on opposite centromere borders (Ma & Bennetzen, 2006; I. Schubert, pers. comm.).

Compared with active centromeres, functionally defined by CenH3, inactive centromeres in maize and wheat lack CenH3 and the kinetochore protein CENP-C (Nasuda *et al.*, 2005; F. Han *et al.*, 2006, 2009; Zhang *et al.*, 2010; Gao *et al.*, 2011). Centromere inactivation is also associated with altered modifications of other histone proteins. Histone H3 phosporylated at serine 10, marking active centromeres during nuclear divisions, is lacking at inactive centromeres of maize (F. Han *et al.*, 2009; Gao *et al.*, 2011) and barley (Houben *et al.*, 1999). Active centromeres are characterized by hypomethylation of CenH3-containing chromatin and hypermethylation of pericentromeric heterochromatin. Centromere inactivation is thought to be associated with cytosine hypermethylation of centromeric sequences (Koo *et al.*, 2011) and loss of pericentromeric heterochromatin – centromere decay (Y. Han *et al.*, 2009; Zhang *et al.*, 2010). Inactive centromeres in maize showed enhanced H3K27me2 and H3K27me3 concentrations (Zhang *et al.*, 2010), marking euchromatin regions in barley (Houben *et al.*, 2003). In fission yeast, inactive centromeres are prevented from reactivation by heterochromatinization and histone deacetylation expanding from the pericentromeric region to the centromere (Sato *et al.*, 2012). Furthermore, experiments using maize dicentric chromosomes have shown that the absence of CenH3 in inactive centromeres led to the loss of Thr133-phosphorylation of histone H2A (Dong & Han, 2012).

It is conceivable that epigenetic changes are immediately or gradually followed by removal of centromere-specific sequences. Exactly how and when centromeric sequences are excised (or reinserted) remains unknown. Intra- and inter-chromosomal UR, the former combined with formation of circular extrachromosomal DNA (eccDNA) molecules, are plausible options (Ventura *et al.*, 2004; Ma *et al.*, 2007a; Gong *et al.*, 2012). Indeed, in humans some centromere inactivations were followed by a partial deletion of the centromere-specific α-satellite associated with CENP-A, which are important for the kinetochore assembly (Stimpson *et al.*, 2010). In budding and fission yeast, originally dicentric chromosomes were stabilized by physical deletion of one centromere (Sato *et al.*, 2012; Stimpson *et al.*, 2012). Data on the recombinational loss of centromeric sequences in plants are scarce. Remnants of pericentromeric heterochromatin at sites of inactive centromeres were interpreted as decaying 'old' or ancestral centromeres (Y. Han *et al.*, 2009; Mandáková *et al.*, 2010a,b; Hu *et al.*, 2011; Wang & Bennetzen, 2012), presumably as a result of gradual sequence removal via UR. In the grass species *B. distachyon*, centromere inactivation was associated with the loss of centromere-specific retrotransposons and rapid turnover of centromeric satellite repeats (Qi *et al.*, 2010). The recent identification of repeatless and repeat-based centromeres in potato (Gong *et al.*, 2012; Wang *et al.*, 2014) is corroborating the concept of rapid turnover of centromere-specific repeats and its role in centromere 'maturation', and possibly also in centromere inactivation.

Centromere repositioning (CR)

Intrachromosomal centromere relocations were classically attributed to pericentric inversions which, according to positions of inversion breakpoints, can relocate a part of pericentromeric heterochromatin (Fransz *et al.*, 2000), the functional core centromere (Lamb *et al.*, 2007) or the entire centromere region (Lysak *et al.*, 2006; Ma *et al.*, 2007b; Wang & Bennetzen, 2012). Pericentric inversions, however, are associated with changes in chromosome collinearity. Hence, intra-chromosomal centromere relocation without alterations in chromosome collinearity cannot be explained by pericentric inversion unless there is a subsequent paracentric inversion that restores collinearity (Lysak *et al.*, 2006; Schubert, 2007). For clades displaying inversions at a low frequency, CR without distortions of chromosome collinearity is a more conceivable alternative (Ventura *et al.*, 2001). CR consists of two interlinked processes, namely 'decay' of old centromere-specific sequences and epigenetic marks, and the emergence of a new functional centromere with appropriate epigenetic characteristics. Analysis of newly formed barley centromeres in wheat–barley hybrid progenies (Nasuda *et al.*, 2005), and of new centromeres on a maize A-chromosome fragment (Fu *et al.*, 2013) and B chromosome (Zhang *et al.*, 2013) showed that a new functional centromere can be formed without centromere-specific sequences, but not without a high DNA methylation level and centromere-specific

epigenetic modifications. Clearly, the mechanics of gradual 'decay' of an old centromere and formation of a new centromere on the same chromosome faces the difficulty of overcoming instability by a transient acentric or a dicentric state.

In plants, CR was claimed for cucumber (*Cucumis sativus*) by comparative bacterial artificial chromosome (BAC)/fosmid mapping on melon (*C. melon*, $2n = 24$) and cucumber ($2n = 14$) chromosomes. Y. Han *et al.* (2009) showed that centromeres of cucumber chromosomes C6 and C7 have changed their positions compared with centromere position on homeologous chromosomes in the ancestral-like melon genome. Identification of CR depends on the quality of sequence assembly and on the density of genetic or cytogenetic markers (e.g. BACs or fosmids). Whereas a new analysis of chromosome collinearity based on next-generation sequencing in cucumber, melon and *C. hystrix* ($2n = 24$) confirmed CR on cucumber chromosome C7, the changed centromere position on chromosome C6 seems to result from multiple chromosome rearrangements (Yang *et al.*, 2014). We presumed CR in *Cardamine rivularis* ($2n = 16$) where the centromere of chromosome CR3 has been relocated to a terminal position, while the ancestral orientation of genomic blocks was preserved (Mandáková *et al.*, 2013). However, the chromosome could have become telocentric and the centromere changed its position as a result of paracentric and pericentric inversions or misrepair of three DSBs in *cis*, with two of them flanking the original centromere position.

Conclusions

The centromere as a site of kinetochore formation ensuring regular chromosome segregation is of the utmost importance for a long-term evolutionary success of both established and new chromosome variants. In taxa with monocentric chromosomes, major chromosome rearrangements have to comply with the dogma of one functional centromere per chromosome ensuring heritability. Reconstruction of chromosome and karyotype evolution considering only 'classical' mechanisms of chromosome rearrangements may be insufficient to explain some genome alterations. Epigenetic chromatin modifications and recombination-mediated indel mechanisms (e.g. excision/insertion of eccDNA) add to the repertoire of chromosome mutations. Reciprocal translocations including nested chromosome insertions are not the only ways of centromere loss during karyotype evolution. Epigenetic loss of the centromere-specific histone H3 variant cenH3 and/or intra-chromosomal recombination can mediate inactivation, loss or relocation of the centromere on a chromosome. Future analyses of newly sequenced and cytogenetically analyzed plant genomes should reveal whether centromere inactivation and loss are rare evolutionary events or play a more substantial role than previously thought, and whether gradual centromere repositioning is a realistic option in the course of karyotype evolution.

Acknowledgements

I would like to thank I. Schubert and J.A. Birchler for critically reading the manuscript, and Y. Weng for valuable comments on the karyotype evolution in Cucurbitaceae. This work was supported by a research grant from the Czech Science Foundation (P501/12/G090) and by the European Social Fund (CZ.1.07/2.3.00/20.0189).

References

Bennetzen JL, Ma J, Devos KM. 2005. Mechanisms of recent genome size variation in flowering plants. *Annals of Botany* **95**: 127–132.

Birchler JA, Gao Z, Sharma A, Presting GG, Han F. 2011. Epigenetic aspects of centromere function in plants. *Current Opinion in Plant Biology* **14**: 217–222.

Birchler JA, Han F. 2013a. Centromere epigenetics in plants. *Journal of Genetics and Genomics* **40**: 201–204.

Birchler JA, Han F. 2013b. Meiotic behavior of small chromosomes in maize. *Frontiers in Plant Science* **4**: 505.

Blower MD, Karpen GH. 2001. The role of Drosophila CID in kinetochore formation, cell-cycle progression and heterochromatin interactions. *Nature Cell Biology* **3**: 730–739.

Cheng F, Mandáková T, Wu J, Xie Q, Lysak MA, Wang X. 2013. Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. *Plant Cell* **25**: 1541–1554.

Cox A, Abdelnour G, Bennett M, Leitch I. 1998. Genome size and karyotype evolution in the slipper orchids (Cypripedioideae: Orchidaceae). *American Journal of Botany* **85**: 681–687.

Cremonini R. 2005. Low chromosome number angiosperms. *Caryologia* **58**: 403–409.

Darlington CD. 1937. *Recent advances in cytology*. London, UK: J&A Churchill.

Dong Q, Han F. 2012. Phosphorylation of histone H2A is associated with centromere function and maintenance in meiosis. *Plant Journal* **71**: 800–809.

Fishman L, Willis JH, Wu CA, Lee Y-W. 2014. Comparative linkage maps suggest that fission, not polyploidy, underlies near-doubling of chromosome number within monkeyflowers (*Mimulus*; Phrymaceae). *Heredity* **112**: 562–568.

Fransz P, Armstrong S, de Jong JH, Parnell LD, Van Drunen C, Dean C, Zabel P, Bisseling T, Jones GH. 2000. Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: structural organization of heterochromatic knob and centromeric heterochromatin. *Cell* **100**: 367–376.

Fu S, Gao Z, Birchler J, Han F. 2012. Dicentric chromosome formation and epigenetics of centromere formation in plants. *Journal of Genetics and Genomics* **39**: 125e130.

Fu S, Lv Z, Gao Z, Wu H, Pang J, Zhang B, Dong Q, Guo X, Wang XJ, Birchler JA *et al.* 2013. *De novo* centromere formation on a chromosome fragment in maize. *Proceedings of the National Academy of Sciences, USA* **110**: 6033–6036.

Gao Z, Fu S, Dong Q, Han F, Birchler JA. 2011. Inactivation of a centromere during the formation of a translocation in maize. *Chromosome Research* **19**: 755–761.

Gong Z, Wu Y, Koblízková A, Torres GA, Wang K, Iovene M, Neumann P, Zhang W, Novák P, Buell CR *et al.* 2012. Repeatless and repeat-based centromeres in potato: implications for centromere evolution. *Plant Cell* **24**: 3559–3574.

Han F, Gao Z, Birchler JA. 2009. Reactivation of an inactive centromere reveals epigenetic and structural components for centromere specification in maize. *Plant Cell* **21**: 1929–1939.

Han F, Lamb JC, Birchler JA. 2006. High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. *Proceedings of the National Academy of Sciences, USA* **103**: 3238–3243.

Han Y, Zhang Z, Liu C, Liu J, Huang S, Jiang J, Jin W. 2009. Centromere repositioning in cucurbit species: implication of the genomic impact from centromere activation and inactivation. *Proceedings of the National Academy of Sciences, USA* **106**: 14937–14941.

Hawkins JS, Proulx SR, Rapp RA, Wendel JF. 2009. Rapid DNA loss as a counterbalance to genome expansion through retrotransposon proliferation in plants. *Proceedings of the National Academy of Sciences, USA* **106**: 17811–17816.

Henikoff S, Ahmad K, Malik HS. 2001. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293**: 1098–1102.

Higgins AW, Gustashaw KM, Willard HF. 2005. Engineered human dicentric chromosomes show centromere plasticity. *Chromosome Research* **13**: 745–762.

Houben A, Demidov D, Gernand D, Meister A, Leach CR, Schubert I. 2003. Methylation of histone H3 in euchromatin of plant chromosomes depends on basic nuclear DNA content. *Plant Journal* **33**: 967–973.

Houben A, Wako T, Furushima-Shimogawara R, Presting G, Kunzel G, Schubert I, Fukui K. 1999. The cell cycle dependent phosphorylation of histone H3 is correlated with the condensation of plant mitotic chromosomes. *Plant Journal* **18**: 675–679.

Howman EV, Fowler KJ, Newson AJ, Redward S, MacDonald AC, Kalitsis P, Choo KH. 2000. Early disruption of centromeric chromatin organization in centromere protein A (Cenpa) null mice. *Proceedings of the National Academy of Sciences, USA* **97**: 1148–1153.

Hu TT, Pattyn P, Bakker EG, Cao J, Cheng JF, Clark RM, Fahlgren N, Fawcett JA, Grimwood J, Gundlach H *et al.* 2011. The *Arabidopsis lyrata* genome sequence and the basis of rapid genome size change. *Nature Genetics* **43**: 476–481.

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P *et al.* 2009. The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics* **41**: 1275–1281.

Jackson RC. 1971. The karyotype in systematics. *Annual Review of Ecology and Systematics* **2**: 327–368.

Jones K. 1998. Robertsonian fusion and centric fission in karyotype evolution of higher plants. *Botanical Review* **64**: 273–289.

Jung S, Cestaro A, Troggio M, Main D, Zheng P, Cho I, Folta KM, Sosinski B, Abbott A, Celton JM *et al.* 2012. Whole genome comparisons of *Fragaria*, *Prunus* and *Malus* reveal different modes of evolution between Rosaceous subfamilies. *BMC Genomics* **13**: 129.

Kalitsis P, Choo KH. 2012. The evolutionary life cycle of the resilient centromere. *Chromosoma* **121**: 327–340.

Khandelwal S. 2008. Chromosome evolution in the genus *Ophioglossum* L. *Botanical Journal of the Linnean Society* **102**: 205–217.

Koo D-H, Han F, Birchler JA, Jiang J. 2011. Distinct DNA methylation patterns associated with active and inactive centromeres of the maize B chromosome. *Genome Research* **21**: 908–914.

Lamb JC, Meyer JM, Birchler JA. 2007. A hemicentric inversion in the maize line knobless Tama flint created two sites of centromeric elements and moved the kinetochore-forming region. *Chromosoma* **116**: 237–247.

Lukaszewski AJ. 1995. Chromatid and chromosome type breakage-fusion-bridge cycles in wheat (*Triticum aestivum* L.). *Genetics* **140**: 1069–1085.

Luo MC, Deal KR, Akhunov ED, Akhunova AR, Anderson OD, Anderson JA, Blake N, Clegg MT, Coleman-Derr D, Conley EJ *et al.* 2009. Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae. *Proceedings of the National Academy of Sciences, USA* **106**: 15780–15785.

Luo MC, Gu YQ, You FM, Deal KR, Ma Y, Hu Y, Huo N, Wang Y, Wang J, Chen S *et al.* 2013. A 4-gigabase physical map unlocks the structure and evolution of the complex genome of *Aegilops tauschii*, the wheat D-genome progenitor. *Proceedings of the National Academy of Sciences, USA* **110**: 7940–7945.

Lysak MA, Berr A, Pecinka A, Schmidt R, McBreen K, Schubert I. 2006. Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. *Proceedings of the National Academy of Sciences, USA* **103**: 5224–5229.

Ma J, Bennetzen JL. 2006. Recombination, rearrangement, reshuffling, and divergence in a centromeric region of rice. *Proceedings of the National Academy of Sciences, USA* **103**: 383–388.

Ma J, Jackson SA. 2006. Retrotransposon accumulation and satellite amplification mediated by segmental duplication facilitate centromere expansion in rice. *Genome Research* **16**: 251–259.

Ma J, Wing RA, Bennetzen JL, Jackson SA. 2007a. Plant centromere organization: a dynamic structure with conserved functions. *Trends in Genetics* **23**: 134–139.

Ma J, Wing RA, Bennetzen JL, Jackson SA. 2007b. Evolutionary history and positional shift of a rice centromere. *Genetics* **177**: 1217–1220.

Malik HS, Henikoff S. 2002. Conflict begets complexity: the evolution of centromeres. *Current Opinion in Genetics & Development* **12**: 711–718.

Malik HS, Henikoff S. 2009. Major evolutionary transitions in centromere complexity. *Cell* **138**: 1067–1082.

Mandáková T, Heenan PB, Lysak MA. 2010b. Island species radiation and karyotypic stasis in *Pachycladon* allopolyploids. *BMC Evolutionary Biology* **10**: 367.

Mandáková T, Joly S, Krzywinski M, Mummenhoff K, Lysak MA. 2010a. Fast diploidization in close mesopolyploid relatives of Arabidopsis. *Plant Cell* **22**: 2277–2290.

Mandáková T, Kovařík A, Zozomová-Lihová J, Shimizu-Inatsugi R, Shimizu KK, Mummenhoff K, Marhold K, Lysak MA. 2013. The more the merrier: recent hybridization and polyploidy in *Cardamine*. *Plant Cell* **25**: 3280–3295.

Martis MA, Klemme S, Banaei-Moghaddam AM, Blattner FR, Macas J, Schmutzer T, Scholz U, Gundlach H, Wicker T, Šimková H *et al.* 2012. Selfish supernumerary chromosome reveals its origin as a mosaic of host genome and organellar sequences. *Proceedings of the National Academy of Sciences, USA* **109**: 13343–13346.

McClintock B. 1939. The behaviour in successive nuclear division of a chromosome broken at meiosis. *Proceedings of the National Academy of Sciences, USA* **25**: 405–416.

Murat F, Xu J-H, Tannier E, Abrouk M, Guilhot N, Pont C, Messing J, Salse J. 2010. Ancestral grass karyotype reconstruction unravels new mechanisms of genome shuffling as a source of plant evolution. *Genome Research* **20**: 1545–1557.

Nasuda S, Hudakova S, Schubert I, Houben A, Endo TR. 2005. Stable barley chromosomes without centromeric repeats. *Proceedings of the National Academy of Sciences, USA* **102**: 9842–9847.

Neumann P, Navrátilová A, Schroeder-Reiter E, Koblížková A, Steinbauerová V, Chocholová E, Novák P, Wanner G, Macas J. 2012. Stretching the rules: monocentric chromosomes with multiple centromere domains. *PLoS Genetics* **8**: e1002777.

Qi L, Friebe B, Wu J, Gu Y, Qian C, Gill BS. 2010. The compact *Brachypodium* genome conserves centromeric regions of a common ancestor with wheat and rice. *Functional & Integrative Genomics* **10**: 477–492.

Salse J. 2012. *In silico* archeogenomics unveils modern plant genome organisation, regulation and evolution. *Current Opinion in Plant Biology* **15**: 122–130.

Sato H, Masuda F, Takayama Y, Takahashi K, Saitoh S. 2012. Epigenetic inactivation and subsequent heterochromatinization of a centromere stabilize dicentric chromosomes. *Current Biology* **22**: 658–667.

Schranz ME, Windsor AJ, Song B-H, Lawton-Rauh A, Mitchell-Olds T. 2007. Comparative genetic mapping in *Boechera stricta*, a close relative of Arabidopsis. *Plant Physiology* **144**: 286–298.

Schubert I. 2007. Chromosome evolution. *Current Opinion in Plant Biology* **10**: 109–115.

Schubert I, Lysak MA. 2011. Interpretation of karyotype evolution should consider chromosome structural constraints. *Trends in Genetics* **27**: 207–216.

Schubert I, Rieger R, Fuchs J. 1995. Alteration of basic chromosome number by fusion–fission cycles. *Genome* **38**: 1289–1292.

Sears ER, Câmara A. 1952. A transmissible dicentric chromosome. *Genetics* **37**: 125–135.

Sierro N, van Oeveren J, van Eijk MJT, Martin F, Stormo KE, Peitsch MC, Ivanov NV. 2013. Whole genome profiling physical map and ancestral annotation of tobacco Hicks Broadleaf. *Plant Journal* **75**: 880–889.

Stimpson KM, Matheny JE, Sullivan BA. 2012. Dicentric chromosomes: unique models to study centromere function and inactivation. *Chromosome Research* **20**: 595–605.

Stimpson KM, Song IY, Jauch A, Holtgreve-Grez H, Hayden KE, Bridger JM, Sullivan BA. 2010. Telomere disruption results in non-random formation of de novo dicentric chromosomes involving acrocentric human chromosomes. *PLoS Genetics* **6**: e1001061.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**: 796–815.

The International Brachypodium Initiative. 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* **463**: 763–768.

The Tomato Genome Consortium. 2013. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **485**: 635–641.

Tobgy HA. 1943. A cytological study of *Crepis fuliginosa, C. neglecta*, and their F1 hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. *Journal of Genetics* **45**: 67–111.

Ventura M, Archidiacono N, Rocchi M. 2001. Centromere emergence in evolution. *Genome Research* **11**: 595–599.

Ventura M, Weigl S, Carbone L, Cardone MF, Misceo D, Teti M, D'Addabbo P, Wandall A, Björck E, de Jong PJ *et al.* 2004. Recurrent sites for new centromere seeding. *Genome Research* **14**: 1696–1703.

Vitte C, Bennetzen JL. 2006. Analysis of retrotransposon structural diversity uncovers properties and propensities in angiosperm genome

evolution. *Proceedings of the National Academy of Sciences, USA* **103**: 17638–17643.

Wang G, Li H, Cheng Z, Jin W. 2013. A novel translocation event leads to a recombinant stable chromosome with interrupted centromeric domains in rice. *Chromosoma* **122**: 295–303.

Wang H, Bennetzen JL. 2012. Centromere retention and loss during the descent of maize from a tetraploid ancestor. *Proceedings of the National Academy of Sciences, USA* **109**: 21004–21009.

Wang L, Zeng Z, Zhang W, Jiang J. 2014. Three potato centromeres are associated with distinct haplotypes with or without megabase-sized satellite repeat arrays. *Genetics* **196**: 397–401.

Wu J, Fujisawa M, Tian Z, Yamagata H, Kamiya K, Shibata M, Hosokawa S, Ito Y, Hamada M, Katagiri S *et al.* 2009. Comparative analysis of complete orthologous centromeres from two subspecies of rice reveals rapid variation of centromere organization and structure. *Plant Journal* **60**: 805–819.

Yang L, Koo DH, Li D, Zhang T, Jiang J, Luan F, Renner SS, Hénaff E, Sanseverino W, Garcia-Mas J *et al.* 2014. Next-generation sequencing, FISH mapping, and synteny-based modeling reveal mechanisms of decreasing dysploidy in *Cucumis*. *Plant Journal* **77**: 16–30.

Zhang B, Lv Z, Pang J, Liu Y, Guo X, Fu S, Li J, Dong Q, Wu H-J, Gao Z *et al.* 2013. Formation of a functional maize centromere after loss of centromeric sequences and gain of ectopic sequences. *Plant Cell* **25**: 1979–1989.

Zhang P, Friebe B, Lukaszewski AJ, Gill BS. 2001. The centromere structure in Robertsonian wheat-rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction. *Chromosoma* **110**: 335–344.

Zhang P, Li W, Friebe B, Gill BS. 2008. The origin of a "zebra" chromosome in wheat suggests nonhomologous recombination as a novel mechanism for new chromosome evolution and step changes in chromosome number. *Genetics* **179**: 1169–1177.

Zhang W, Friebe B, Gill BS, Jiang J. 2010. Centromere inactivation and epigenetic modifications of a plant chromosome with three functional centromeres. *Chromosoma* **119**: 553–563.