Interpretation of karyotype evolution should consider chromosome structural constraints

Ingo Schubert[1] and Martin A. Lysak[2]

[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D-06466 Gatersleben, Germany
[2] Department of Functional Genomics and Proteomics, and CEITEC, Masaryk University, CZ-625 00 Brno, Czech Republic

Comparative genetics, genomics and cytogenetics provide tools to trace the evolutionary history of extant genomes. Yet, the interpretation of rapidly increasing genomic data is not always done in agreement with constraints determined by chromosome structural features and by insights obtained from chromosome mutagenesis. The terms 'non-reciprocal chromosome translocation', 'chromosome fusion' and 'centromere shift' used to explain genomic differences among organisms are misleading and often do not correctly reflect the mechanisms of chromosome rearrangements underlying the evolutionary karyotypic variation. Here, we (re)interpret evolutionary genome alterations in a parsimonious way and demonstrate that results of comparative genomics and comparative chromosome painting can be explained on the basis of known primary and secondary chromosome rearrangements. Therefore, some widespread terms used in comparative and evolutionary genomics should be either avoided (e.g. nonreciprocal translocation) or redefined (e.g. chromosome fusion and centromere shift).

Interpretation of genomic data needs to be in agreement with chromosome constraints

Nuclear genomes are contained within chromosomes representing genetic linkage groups. The number, size and shape of chromosomes, which constitute the karyotype (see Glossary) of an organism, vary considerably among groups of eukaryotes. The increasing number of sequenced genomes has revealed that most higher eukaryote genomes contain between approximately 14000 and 40000 protein coding genes. By contrast, nuclear genome sizes vary by more than four orders of magnitude from approximately 12 Mb in yeast [1] up to 400000 Mb in dinoflagellates [2]. Accordingly, the size of linear metaphase chromosomes spans a range from <1 mm to some dozen micrometers. The number of chromosome pairs can also vary widely, from 1 (in an ant [3]) to approximately 700 in the fern genus *Ophioglossum* [4]. Whereas genome size and chromosome numbers are rather stable in some phylogenetic clades, they vary considerably in others [5].

Several mechanisms responsible for the variation in size, shape and number of chromosomes, as well as in DNA content between organisms, are recognized. Chromo-

somes change their size and shape by gain (i.e. insertion or duplication) or loss (i.e. deletion) of DNA, or through rearrangements within or between chromosomes. Chromosome rearrangements can also increase or decrease the number of chromosomes, a phenomenon called ascending or descending dysploidy, respectively [6]. In addition, chromosome numbers can be altered by ploidy mutations involving the entire complement (polyploidy) or individual chromosomes (aneuploidy).

The evolutionary history of a karyotype is often difficult to determine, especially for older events. With time, the accumulation of chromosome rearrangements will obscure the exact identity, number and order of events that have occurred along a lineage leading to extant karyotypes. There are, however, techniques to help reconstruct this blurred history. Comparative chromosome painting [7,8] has proved to be useful for tracing karyotype evolution in mammals (reviewed in [9]) and in plants (e.g. in Brassicaceae [10–13]). Alternatively, karyotype evolution can be

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Glossary

Acrocentric: bi-armed chromosome with asymmetric arm length, including one very short arm.
Aneuploidy: single chromosome types are less (e.g. $2n - 1$, hypoploid) or more frequent (e.g. $2n + 1$, hyperploid) than chromosome pairs.
Chromosome painting: fluorescent *in situ* hybridization (FISH) with chromosome or chromosome region-specific probes to the chromosome complement of a given species or a closely related one.
Dysploidy: decrease or increase of chromosome number in connection with chromosome rearrangements; not by addition or loss of single chromosomes as in aneuploidy. Therefore, dysploidy is also called pseudoaneuploidy.
Homeologues: chromosomes of related extant species that share a similar gene content in the same (syntenic) order.
Karyotype: entire chromosome complement of an organism.
Linkage group: combination of sequences (in a defined order) that segregate together during meiosis because they are located on the same chromosome.
Metacentric: chromosome with approximately equal-sized chromosome arms.
Monocentric: chromosome with one centromere (attachment site of spindle fibres during nuclear divisions) that appears as a primary constriction. This is in contrast to dicentric chromosomes with two centromeres and poly- or holocentric chromosomes on which spindle fibres attach along almost the entire chromosome length.
Palaeogenomics: comparison of genetic, genomic and cytogenetic data of extant species to interpret their evolutionary origin from tentative ancestral genomes.
Robertsonian rearrangements (i.e. fusion–fission cycle): symmetric or asymmetric translocation between the centric ends of two telocentric or acrocentric chromosomes forming a metacentric chromosome ('centric fusion'), or splitting of a metacentric chromosome into two telocentric chromosomes ('centric fission'); see Figure 3d, main text.
Telocentric: quasi one-armed chromosome with a short arm (presumably) consisting only of the telomere flanking the centromere on one side.

Corresponding author: Schubert, I. (schubert@ipk-gatersleben.de).

studied by using comparative genetic and genomic analyses. Palaeogenomics, which is an integrated approach combining genetic and cytogenetic maps, EST data sets [14,15] and whole-genome sequences [16–20], enables researchers to trace the evolutionary history of genomes within a phylogenetic framework. It also helps to elucidate whole-genome duplications and genome reshuffling that have led to extant chromosome complements (Box 1).

A re-interpretation is necessary in cases when comparative genomics neglected the mechanics of chromosome rearrangements; for example, by postulates that disagree with observations from chromosome mutagenesis. Our aim here is to bring genomic interpretation into accordance with chromosome constraints. We focus on mechanisms that underlie karyotypic changes as a basis for interpreting comparative genomic data and for elucidating the evolutionary origin of extant karyotypes.

Mechanisms altering chromosome complements are diverse but do not include 'non-reciprocal translocation'

During the course of evolution, a karyotype can be altered by changing the number, structure and composition of its chromosomes. Ploidy mutations that multiply the entire chromosome complement of an organism have been observed in all eukaryotic phyla. When polyploidization has occurred recently in evolutionary time, the corresponding multiplication of chromosome number is still recognizable (neopolyploidy). Polyploidization followed by diploidization including chromosome number reduction (mesopolyploidy) is detectable by using comparative genomics and chromosome painting (e.g. as done for several Brassicaceae species [10,13,21]). Ancient polyploidization events, blurred by many subsequent alterations of the genome (palaeopolyploidy) can only be revealed in extant diploid taxa by bioinformatic searches for orthologous and

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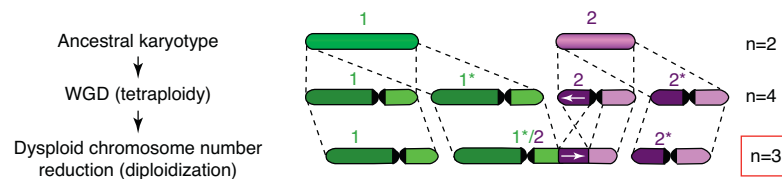### Box 1. Analysis of the evolutionary history of an extant genome

Palaeogenomics analyses to explore the evolutionary history of an extant genome under consideration of chromosomal constraints should comprise three components complementing each other: (i) elucidation of the phylogenetic relationship between the extant taxon of interest and its presumably closest relatives to get a hint to the direction of the evolution. This requires the comparative analysis of informative sequences suitable to estimate the phylogenetic distance between extant taxa and possibly the length of time since divergence from the last common ancestor by means of base substitutions; (ii) elucidation of syntenic relationship between linkage groups (chromosomes) of

the extant genomes to get an indication of potential ancestral whole-genome duplication (polyploidy) and/or chromosome rearrangements. Dense genetic linkage maps or draft whole-genome sequences of the taxa to be compared are required to establish a syntenic relationship and to estimate the redundancy of syntenic block between the extant genomes; (iii) reconstruction of chromosome rearrangements that are most likely to have shaped the extant genomes. Comparative chromosome painting, with suitable BAC contigs for instance, can reveal or specify the actual rearrangement that is most likely to have lead to the karyotype of the extant genomes (Figure I).
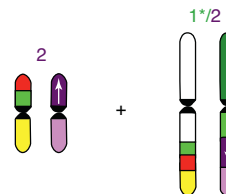

(a) Phylogenetic analysis
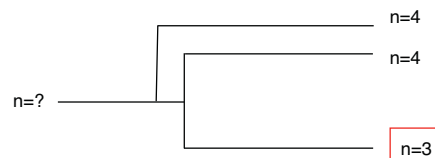
(b) Comparative genetics and/or genomics

(c) Comparative cytogenetics by chromosome painting

TRENDS in Genetics

Figure I. Three approaches to explore the evolutionary history of an extant genome comprehensively. **(a)** Phylogenetic analysis via comparison of informative sequences reveals that the taxon with $n = 3$ chromosomes is apparently derived from an ancestor with $n = 4$. **(b)** Comparative genetic mapping and/or genomics reveals conserved synteny within and between linkage groups (i.e. chromosomes) of extant taxa with $n = 4$ and $n = 3$, respectively. This analysis indicates that both extant taxa descended from an ancestor with $n = 2$ chromosomes and, after whole-genome duplication towards $n = 4$, descending dysploidy has led to a karyotype of $n = 3$. **(c)** Comparative cytogenetics by chromosome painting suggests the most probable origin of the extant karyotype with $n = 3$ to be a whole-arm pericentric inversion in chromosome 2 and subsequent reciprocal translocation between chromosomes 1* and 2, resulting in chromosome 1*/2 and a small product, 2/1*, which got lost (see Figure 3a, main text). Abbreviation: WGD, whole-genome duplication.

paralogous sequence markers (reviewed in [22]). Although polyploid genomes increase the cost of replication, they also provide evolutionary advantages, because they present a basis for speciation. Gene duplicates can acquire new functions and chromosome rearrangements that lead to deletions (see below) that are usually lethal in diploid genomes can be tolerated in neo- and mesopolyploid genomes.

Structural chromosome alterations are the result of primary or secondary rearrangements. Primary rearrangements are the outcome of illegitimate recombination during double-strand break (DSB) repair, either via direct joining of ends between different DSBs, or through recombination with ectopic (instead of allelic) homologous sequences. The frequent use of ectopic homologous sequences as template for recombination repair explains why primary rearrangements have breakpoints preferentially within heterochromatic regions enriched in similar repetitive sequences [11,23].

Primary chromosome rearrangements are: insertion, deletion or duplication, peri- or paracentric inversion, and intra- or interchromosomal reciprocal translocation (Figure 1). Deletions are tolerated only in polyploids or when dispensable sequences are involved [24,25]. Contrary to reciprocal translocation, during which chromosomes exchange segments mutually, in non-reciprocal translocation a chromosome segment is transferred in a unidirectional manner from one chromosome to another. Duplicated chromosome segments on different chromosomes have also been hypothesized to result from non-reciprocal translocation. Non-reciprocal exchange of microscopically detectable chromosome segments has, however, never been demonstrated experimentally and suspect cases can be interpreted as unbalanced segregation after reciprocal translocation. Indeed, unbalanced segregation from reciprocal translocations can yield daughter nuclei with duplications and deletions, mimicking 'non-reciprocal' translocations (Figure 1d, bottom right). Cells with nuclei containing deletions are usually counter-selected, but can survive when deletions occur in polyploid backgrounds or in some tumours [26].

Gene conversion [27–29], between homeologous chromosomes of polyploid species, can transfer homeologous sequences in a non-reciprocal manner [27–29]. However, gene conversion should not be misinterpreted as non-reciprocal translocation. Gene conversion rather represents a variant of DSB repair during which, by transient invasion of break-ends into a sequence-related double helix and subsequent replicative elongation of break-ends (to bridge the DSB), relatively short regions are copied into the broken double helix (similar to that shown in Figure 1b, bottom left).

Structural chromosome alterations can additionally arise as secondary chromosome rearrangements (Figure 2; reviewed in [30]). Such rearrangements can arise in organisms that are doubly heterozygous for two primary rearrangements (translocations and/or inversions), if one chromosome is involved in both. Meiotic crossing over between homologous regions of rearranged chromosomes, which differ in the regions distal to the cross over, leads to gametes with a new karyotype and to complementary gametes displaying a re-established wild-type chromosome complement (Figure 2a). Depending on the type of primary rearrangements involved, unbalanced gametes can also arise, harbouring duplications as well as deletions and mimicking non-reciprocal translocation (Figure 2b) [31,32].

In summary, experimentally observed ploidy mutations, as well as primary and secondary chromosome rearrangements, are sufficient to explain evolutionary karyotype alterations. Even the interpretation of unbalanced karyotypes does not require the assumption of non-reciprocal translocations (see also below).

'Chromosome fusion' is the result of reciprocal translocation

Geneticists speak of chromosome fusion when all genetic markers belonging to two ancestral genetic linkage groups segregate as a single linkage group in a derived species. Similarly, chromosome painting probes can cover two chromosomes in an ancestral karyotype and only one in a more derived species. Such cases do not necessarily represent chromosome fusions. A simple fusion of intact chromosomes is unlikely because telomeres distinguish natural ends of linear chromosomes from break-ends and prevent fusion of natural chromosome ends [33]. True fusion of chromosome ends (e.g. forming ring chromosomes) can occur in telomerase mutants [34]; however, the karyotypes of telomerase mutants are unstable unless the mutation becomes compensated by an alternative telomere elongation mechanism stabilizing the chromosome ends. Chromosome fusion *sensu stricto* further implies that no loss of chromatin occurs and the process is reversible. Usually, however, the 'fused' chromosomes have only one centromeric region and no large internal telomeric sequence arrays, indicating an irreversible symmetric reciprocal translocation, rather than a fusion, as the causative process. Furthermore, in the first metaphase after exposure to a genotoxic compound or ionizing irradiation, ligations between entire chromosomes have so far not been reported to occur among the structural chromosome mutations. Therefore, the term 'chromosome fusion' has to be understood as a substitute for reciprocal translocation combining two linkage groups within the larger of two translocation products.

In so-called 'end-to-end fusion', a telo- or acrocentric chromosome (with no essential genes in its short arm) undergoes symmetric reciprocal translocation with another chromosome with breakpoints close to the centromere in the long arm of the telo- or acrocentric chromosome, and close to one arm end in the other chromosome (Figure 3a). The large translocation product therefore combines most of both chromosomes, whereas the second, small product comprises the centromere of the telo- or acrocentric chromosome plus two telomeres. Such small chromosomes, when free of essential genes, are often lost by unstable transmission through meiosis [35–37]. If two metacentrics are to be combined into one, at least one of them has to become telo- or acrocentric; for example, via a pericentric inversion (with one breakpoint close to the centromere and the other close to the opposite arm end) before reciprocal translocation (Figure 3a). Such inversions are detectable in dense genetic and/or cytogenetic maps [11,12,38]. Chromosome colinearity can be restored by a second paracentric
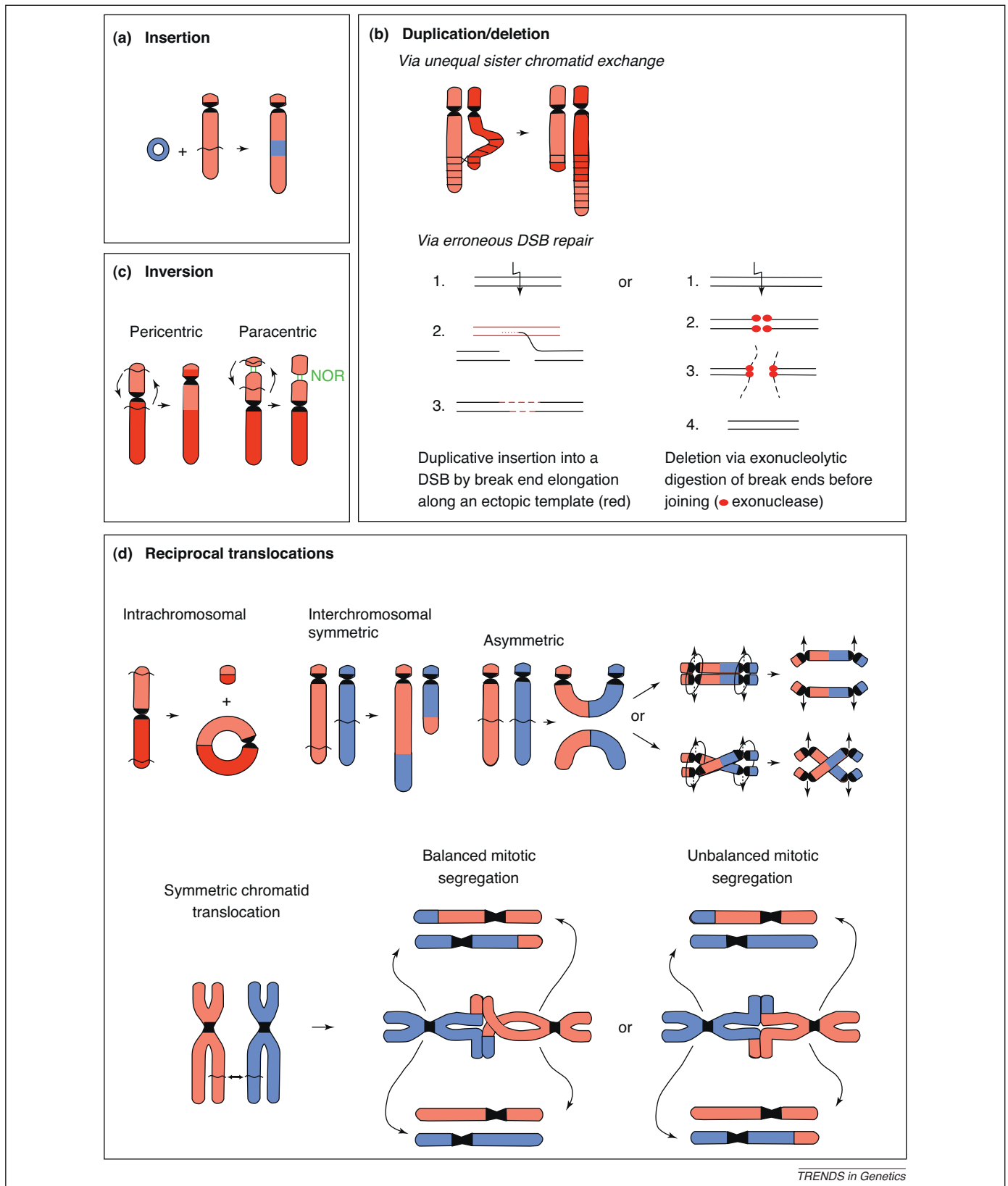
Figure 1. Primary structural chromosome rearrangements. **(a)** Insertion of alien or endogenous sequences through integration of extrachromosomal circular DNA [59,60]. **(b)** Deletions or duplications via unequal sister chromatid exchange or unequal crossing over at meiosis (top), or via erroneous DSB repair (bottom) [61,62]. **(c)** Pericentric inversion with breakpoints on either side of the centromere (black constricted area) and paracentric inversion with both breakpoints within the same chromosome arm. A nucleolus-organizing region (NOR) is shown to illustrate the effect of paracentric inversion on its position. **(d)** Intrachromosomal reciprocal translocations resulting in a ring chromosome and an acentric fragment, and interchromosomal translocations that can be either symmetric and yield monocentric products, or asymmetric, resulting in a dicentric chromosome and an acentric fragment. The products of asymmetric reciprocal translocations are usually unstable: the acentric fragment gets lost because of the lack of a centromere; the instability of dicentric fragments increases with the distance between the centromeres because of the increasing risk that sister chromatids twist between centromeres, resulting in anaphase bridges (top right), which subsequently get disrupted. Symmetric chromatid translocations might be subject to balanced or unbalanced mitotic segregation, the latter yielding nuclei with deletions and duplications, respectively (bottom right). Unbalanced segregation is usually lethal, but, if survived, mimics non-reciprocal translocation.
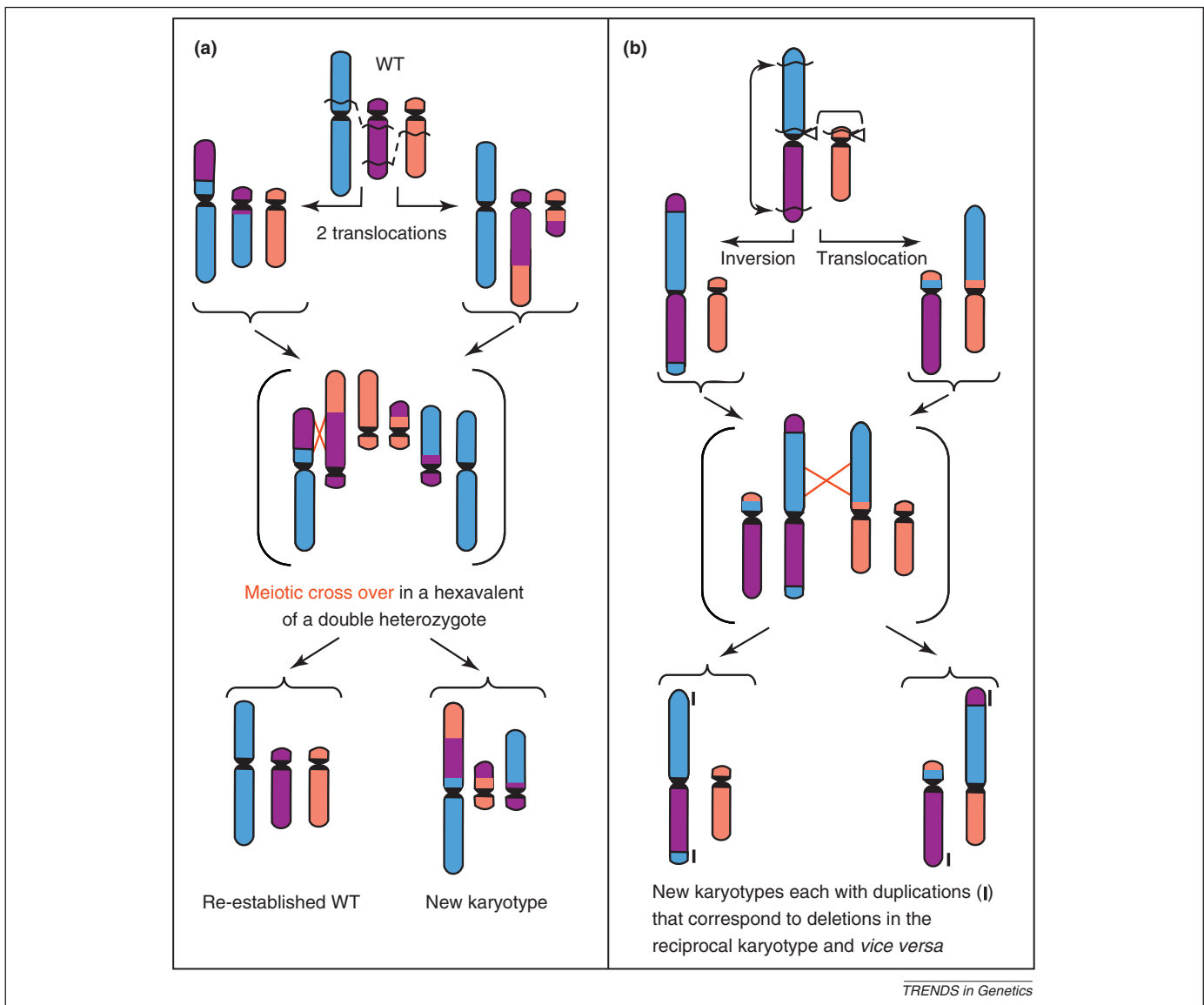
Figure 2. Secondary chromosome rearrangements. **(a)** Two translocations involving three wild-type (WT) chromosomes in different individuals lead to hexavalents (in brackets) during meiosis in double heterozygous individuals. Crossing over (red) involving homologous regions flanked by non-homologous regions generates a new karyotype and re-establishes the WT chromosome complement. **(b)** If one chromosome is involved in an inversion in one individual and in a translocation in another individual, meiotic crossing over involving homologous regions flanked by non-homologous regions generates two new karyotypes, each harbouring complementary duplication and deletions, respectively (bottom).

inversion involving the pericentrically inverted arm [11]. Asymmetric reciprocal translocation between the ends of two metacentrics (mimicking end-to-end fusion) can also yield a large stable chromosome if one centromere becomes lost or inactivated [13]. Circumstantial evidence for all variants that mimic an end-to-end chromosome fusion has been obtained by comparative chromosome painting [11–13].

'Insertional' or 'nested chromosome fusion', where an ancestral linkage group is on both ends flanked by markers of the chromosome arms of a second ancestral linkage group, also needs reinterpretation (Figure 3b). Such fusion types have been detected in several grass species [19,39–43] and less frequently in Brassicaceae [11]. Instead of 'fusion' of the 'insertion' chromosome into a single centromeric DSB of the 'recipient' chromosome, simultaneous breaks at both arm ends of the insertion chromosome

and around the centromere of the recipient chromosome can, via mis-repair, result in an asymmetric insertional translocation, adding the arms of the recipient chromosome to the arms of the insertion chromosome. The centromere of the recipient chromosome (or separated parts of the recipient centromere) must be inactivated to stabilize the new 'fusion' chromosome and the small acentric translocation product gets lost. Inactivation of centromeres has been observed [44,45]. When, however, the recipient chromosome has a break on either side of its centromere, a symmetric reciprocal translocation can yield two monocentric products: the large one spanning both original linkage groups and a smaller one comprising the centromere of the recipient chromosome and the telomeres of the inserted chromosome. The small product is prone to loss during meiosis [29]. It is hard to prove whether the centromere of the recipient chromosome (or its split portions)
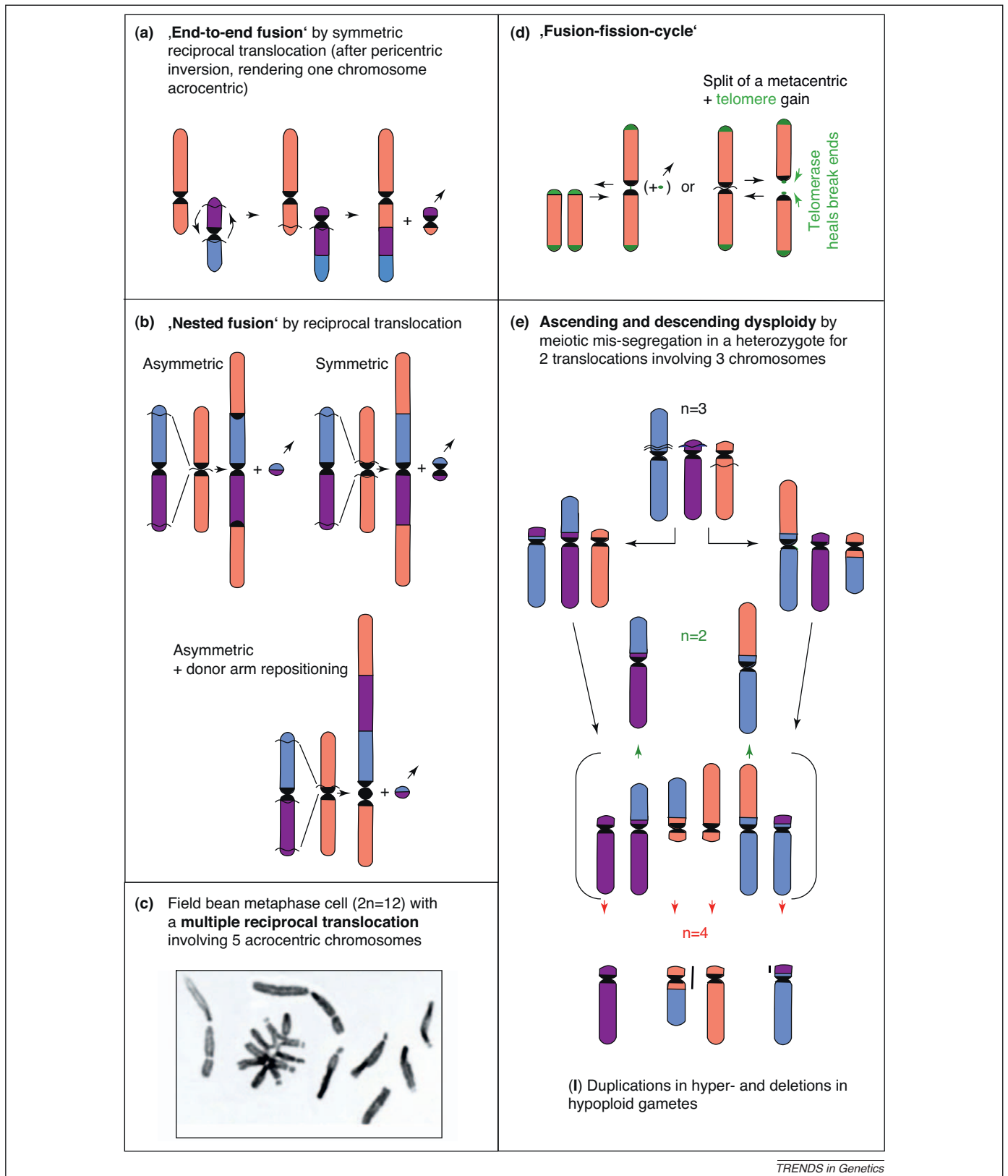
Figure 3. Interpretation of dysploid alterations of chromosome number. **(a)** 'End-to-end fusion' by symmetric reciprocal translocation (after pericentromeric inversion rendering one chromosome acrocentric) yields a large and a small monocentric product; the latter prone to getting lost during meiosis. **(b)** 'Nested fusion' by asymmetric (top left), symmetric (top right) or asymmetric reciprocal translocation with donor arm re-positioning (bottom) [39] might appear depending on the number of interacting DSBs; the small acrocentric fragments are lost during mitosis and the small centric fragment might get lost during meiosis. **(c)** Multiple chromatid translocation involving five acrocentric chromosomes in a complete first metaphase of the field bean *Vicia faba* after treatment with an S phase-dependent mutagen. **(d)** 'Fusion–fission cycle' (Robertsonian rearrangements) can reversibly alter the chromosome number by asymmetric reciprocal translocation involving centric chromosome ends of telocentric chromosomes and yielding a meta(di)centric chromosome and an acentric fragment consisting of telomeric sequence arrays (green). A DSB within the telomeric sequences between the two centromeres of the large translocation product can re-establish two stable telocentric chromosomes (left), as proven for the field bean [46]. Alternatively, DSB within a monocentric bi-armed chromosome can yield stable telocentric chromosomes when telomeric sequences are added to the break-ends (right). These telocentric chromosomes could again 'fuse' by symmetric (irreversible) or asymmetric (reversible) reciprocal translocation. **(e)** Simultaneously ascending and descending

was deleted after inactivation, or whether the respective chromosome was monocentric from the beginning owing to symmetric translocation. In some cases of 'nested' chromosomes of *Brachypodium distachyon*, centromeric (but not telomeric; T. Wicker, pers. communication) sequences were found to flank the insertion chromosome [19]. It remains elusive whether these centromeric sequences (much less copious than in active centromeres) represent remnants of a gradually inactivated recipient centromere or were insufficient to establish a functional centromere immediately after translocation. Theoretically, the two arms of the recipient chromosome could have been translocated to the nested chromosome arm ends via two subsequent events. However, it is hard to explain why the second event again involved the same two chromosomes in so many cases [19,39,40]. It is easier to assume that multiple break-ends interact when the territories of the involved chromosomes are in close vicinity. Complex chromosome rearrangements resulting from multiple breaks that occur simultaneously can indeed be observed in the first nuclear division after their origin (Figure 3c). A simultaneous translocation of broken insertion chromosome arm ends to break-ends of the same recipient chromosomes bears a lower risk for the appearance of unbalanced gametes (to be selected against) during meiotic segregation, than does a simultaneous translocation to different recipient chromosomes.

Summarizing, one can consider linkage groups to be fused, but a simple fusion of chromosome ends is not compatible with the end-protecting function of telomeres. Reciprocal translocation events therefore provide a more probable interpretation.

Translocation-based dysploid chromosome number alterations are experimentally proven

Asymmetric reciprocal translocation between the centric ends of two telocentric chromosomes results in a dicentric 'fusion' chromosome and a dispensable acentric fragment consisting of telomeric sequences (descending dysploidy). In the course of a chromosomal 'fusion–fission cycle' (Robertsonian rearrangements [6]), stable centric fission products (ascending dysploidy) can arise from a break within the remaining telomere sequence array that separates the centromeres of the 'fusion' dicentric ([46], Figure 3d, left). By contrast, fission within a centromeric region of a 'normal' monocentric chromosome requires a split of the original centromere into two functional fragments and the addition of telomeres for survival of the resulting telocentric chromosomes (Figure 3d, right). Although telomerase prefers pre-existing telomere repeats to prime the elongation of a telomere array, recombination-dependent 'telomere capture' [47] and *de novo* synthesis of telomeres (e.g. in *Tetrahymena*, [48], or in wheat, [49]) were reported to be involved in 'chromosome healing'. Internal telomere repeats that result from ancient inversions with one breakpoint in a telomeric array could also prime telomere elongation when positioned at a breakpoint.

As an alternative to the 'fusion–fission cycle', the occurrence of two translocations between three chromosomes (one of the three involved in both translocations) with all breakpoints close to the centromeres can result in simultaneous ascending and descending dysploidy (Figure 3e). In individuals double-heterozygous for both translocations, a hexavalent is formed during meiotic chromosome pairing. At a low frequency, the two metacentric translocation chromosomes of the hexavalent segregate to one pole and the four acrocentric chromosomes to the other. Consequently, gametes containing the acrocentric translocation chromosomes have one chromosome more than do the parental lines and a duplication of at least one centromere plus two terminal regions, whereas gametes with the metacentric translocation chromosomes have one chromosome less and the corresponding deletions. When gametes of the same dysploid karyotype fuse, the chromosome number can increase or decrease simultaneously in a homozygous fashion, provided the accompanying duplications and deletions can be tolerated. This dysploidy mechanism has been experimentally proven in *Vicia faba* [50]. Similar mechanisms have been suggested elsewhere [37]. The increase in chromosome number from $n = 10$ to $n = 12$ postulated for a common (tetraploid) ancestor of cereals [14] could be explained in the same way. After whole-genome duplication events, a broad range of chromosome rearrangements can be tolerated [13], and dysploidies, including deletions or duplications, are likely to become fixed but are difficult to interpret later on as, for example, the descending dysploidy leading to the extant maize genome [14,43,51,52]. The more distant from the centromere the breakpoints in the translocation chromosomes of the double heterozygous individuals are, the larger the duplicated (and the corresponding deleted) regions in the dysploid progeny karyotypes are. The resulting segmental duplications or deletions can again give the erroneous impression of 'non-reciprocal translocations'.

Epigenetic *de novo* formation of a centromere without specific sequence requirement (as described for humans [53], *Drosophila* [54] and barley [44]), could also result in a dysploid increase in chromosome number when occurring in acentric fragments. Although several cases of *de novo* formation of a regular centromere have been described, such an event has so far not been observed in *statu nascendi* (i.e. in the first metaphase after genotoxin exposure). The reason could be that epigenetic *de novo* centromere formation is very rare and, therefore, most acentric fragments get lost.

Experimentally proven Robertsonian rearrangements [46] and numerical changes by meiotic mis-segregation in double heterozygous carriers of suitable reciprocal translocations [50] offer an adequate explanation for dysploid chromosome number alteration. Terms that denote hitherto unproven processes, such as chromosome fusion and non-reciprocal translocation, should therefore be avoided.

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dysploid karyotypes can be the result of mis-segregation from meiotic hexavalents (in brackets) of individuals doubly heterozygous for two translocations involving three chromosomes (one of them metacentric) when the two metacentric translocation chromosomes segregate to one pole and the four acrocentric chromosomes to the other (as proven for the field bean [50]). The hypoploid gametes harbour small deletions and the hyperploid gametes the corresponding duplications (bars), mimicking non-reciprocal translocations.
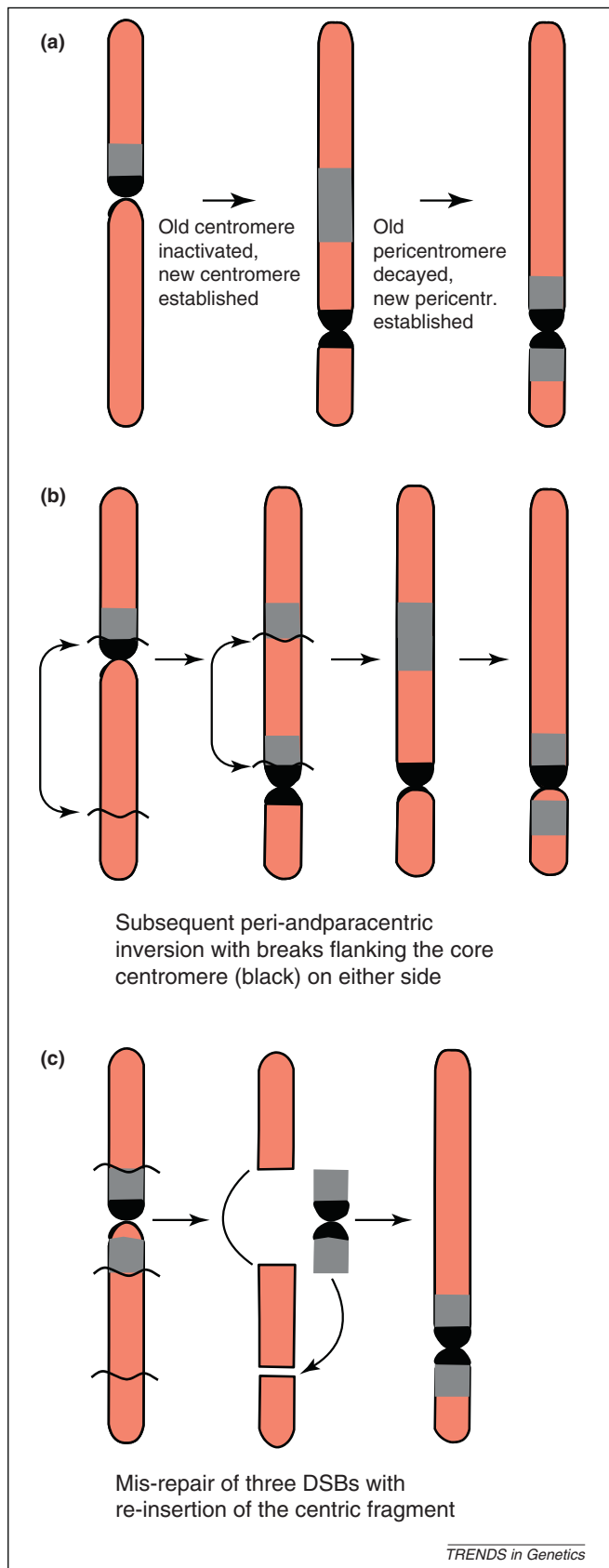
Figure 4. Alternative interpretations of positional centromere shift. **(a)** A centromere at the old position becomes inactivated and a new centromere gets established at another position; subsequently, the old pericentromere (grey) decays and a new pericentromeric sequence accumulates at the new centromere position. **(b)** Subsequent peri- and paracentric inversions, each with a break flanking the core centromere (black) on either side can be followed by decaying of pericentromeric arrays at old and re-establishing at new centromeric positions. **(c)**

Intrachromosomal centromere shifts can be explained by known primary rearrangements

The positional shift of a centromere means the loss at one position in favour of an appearance at a new position on the same chromosome without changing the sequence colinearity between the old and the new centromere position (Figure 4a). Such centromere repositioning can change the arm ratio and, thus, the shape of the corresponding chromosome; however, the underlying mechanisms have remained obscure. In the case of a centromere shift in a monocentric chromosome, it is unclear how the corresponding chromosome resolves the problem of having either no active or two centromeres during the transition phase (i.e. one no longer active and one just-arising centromere). Centromere shifts assuming a gradual decay at the old and *de novo* appearance of a centromere at the new position are claimed for primates [55], for the genus *Equus* [56] and for homeologous chromosomes of cucumber and melon [57]. However, the observed positional shift of centromeres between cucumber chromosome 6 and melon chromosome 1 is probably the result of a reciprocal translocation combining two melon chromosomes into one cucumber chromosome (see Figure 3a and [58]). The centromere shift in another homeologue between cucumber and melon, could be explained as a result of two subsequent inversions, one pericentric and the other paracentric, each with a breakpoint flanking the 'old' core centromere but on opposite sides (Figure 4b). Alternatively, intrachromosomal ligation between the two distal break-ends of centromere-flanking DSBs and simultaneous insertion of the resulting centric fragment product into another DSB on the same chromosome (Figure 4c) could explain centromere shifts. Thus, two subsequent inversions or a transposition-like re-insertion seem to be plausible and parsimonious explanations for centromere shifts in monocentric chromosomes, as long as identical or similar sequences occur at old and new core centromere positions. Only if no centromeric sequences typical for the given species are detectable at the new centromere position, is a shift by epigenetic 'de novo' centromere formation probable; however, it is still necessary to explain why and how the old centromere became inactive or deleted.

Concluding remarks

We have shown that several abundant differences that discriminate extant genomes can be explained on the basis of known and inducible primary and spontaneous secondary chromosome rearrangements that had to pass mitotic and meiotic divisions, without assuming unproven processes. For segmental duplications or deletions, it is not necessary to claim 'non-reciprocal' translocations because unbalanced segregation in progenies of heterozygous translocation carriers, or secondary chromosome rearrangements offer reasonable explanations. The different types of 'chromosome fusion' can all be explained by reciprocal translocations based on different numbers of simultaneously occurring DSBs and do not require unlikely

The distal ends of centromere-flanking DSBs undergo intrachromosomal ligation and the centric fragment gets re-inserted into another DSB on the same chromosome. **(b)** and **(c)** do not have to pass a potential dicentric or acentric state, whereas as **(a)** does.

interaction of telomeres with break-ends. In some cases, however, differences in genome structure are highly complex (caused by several subsequently occurring events) and, thus, can be explained either only tentatively or equally well by alternative mechanisms. Nevertheless, we expect that our approach will provide a satisfying interpretation for future findings of comparative genetics, genomics and cytogenetics.

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