

VIEWPOINT

Holocentric chromosomes: from tolerance to fragmentation to colonization of the land

František Zedek* and Petr Bureš

Department of Botany and Zoology, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic

**For correspondence. E-mail fzedek@gmail.com*

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- **Background** The dispersed occurrence of holocentric chromosomes across eukaryotes implies they are adaptive, but the conditions under which they confer an advantage over monocentric chromosomes remain unclear. Due to their extended kinetochore and the attachment of spindle microtubules along their entire length, holocentric chromosomes tolerate fragmentation; hence, they may be advantageous in times of exposure to factors that cause chromosomal fragmentation (clastogens).
- **Scope** It is shown that holocentric organisms may, indeed, thrive better than monocentric organisms under clastogenic conditions and that such conditions of various duration and intensity have occurred many times throughout the history of Earth's biota. One of the most important clastogenic events in eukaryotic history, in which holocentric chromosomes may have played the key role, was the colonization of land by plants and animals half a billion years ago. In addition to arguments supporting the anticlastogenic hypothesis of holocentric chromosomes and a discussion of its evolutionary consequences, experiments and analyses are proposed to explore this hypothesis in more depth.
- **Conclusions** It is argued that the tolerance to clastogens explains the origin of holocentric lineages and may also have far-reaching consequences for eukaryotic evolution in general as exemplified by the potential role of holocentric chromosomes in terrestrialization.

Key words: Clastogens, cosmic radiation, chromosomal fragmentation, desiccation, gamma radiation, herbivory, holokinetic chromosomes, land plants, monocentric chromosomes, terrestrialization, ultraviolet radiation, Zygnematophyceae.

WHAT ARE HOLOCENTRIC CHROMOSOMES GOOD FOR?

Chromosomes in eukaryotes are nucleoprotein packages whereby DNA is faithfully transmitted across cell and organismal generations. In each cell division, spindle microtubules grab chromosomes by their 'handles' and pull them to daughter cells. In eukaryotes with monocentric chromosomes, this handle – the kinetochore – is formed in a centromeric region. Some eukaryotic lineages, however, have independently evolved holocentric chromosomes that form the kinetochore along their entire length (Figs 1 and 2; Mola and Papeschi, 2006; Melters *et al.*, 2012; Bureš *et al.*, 2013). Although the repeated origin of holocentric chromosomes by convergent evolution implies that holocentrism is adaptive, the conditions under which holocentrism may have provided a selective advantage are unclear.

The extended kinetochore of holocentric chromosomes may suppress the meiotic drive of centromeric repeats and its negative consequences (Talbert *et al.*, 2008; Malik and Henikoff, 2009; Zedek and Bureš, 2016). However, the hypothesis of centromere drive suppression only explains the evolution of chromosomal holocentrism in meiosis, not in mitosis (Zedek and Bureš, 2016). Moreover, lineages exist that are holocentric only in mitosis but not in meiosis (reviewed by Marques and Pedrosa-Harand,

2016). Conceivably, the origin of mitotic holocentrism could sometimes entail meiotic holocentrism – perhaps due to a shared machinery between these two types of cell division – which could then be adopted for centromere drive suppression, or mitotic and meiotic holocentrism may be two unrelated adaptations (Zedek and Bureš, 2016). In any case, the repeated evolution of mitotic/somatic holocentrism (Mola and Papeschi, 2006; Melters *et al.*, 2012; Bureš *et al.*, 2013), a feature that all currently known holocentric organisms have in common (Marques and Pedrosa-Harand, 2016), requires an explanation of its own.

Mitosis is central to development, and any disruptions of this process may reduce an individual's fitness and chances of surviving to a reproductive age. The key to the adaptive value of mitotic holocentrism may therefore lie in the tolerance of holocentric chromosomes to fragmentation due to their extended kinetochore (Mandrioli and Manicardi, 2012; Bureš *et al.*, 2013). During cell divisions, all the fragments of holocentric chromosomes retain their kinetic activity and are normally transmitted to daughter cells, each of which receives half of the genetic material (Fig. 1; Nordenskiöld, 1963; Murakami and Imai, 1974; Sheikh *et al.*, 1995; Carpenter *et al.*, 2005; Jankowska *et al.*, 2015). In fact, the regular inheritance of chromosomal fragments is considered strong evidence of holocentrism (reviewed by Mola and Papeschi, 2006; Melters *et al.*, 2012; Bureš *et al.*,

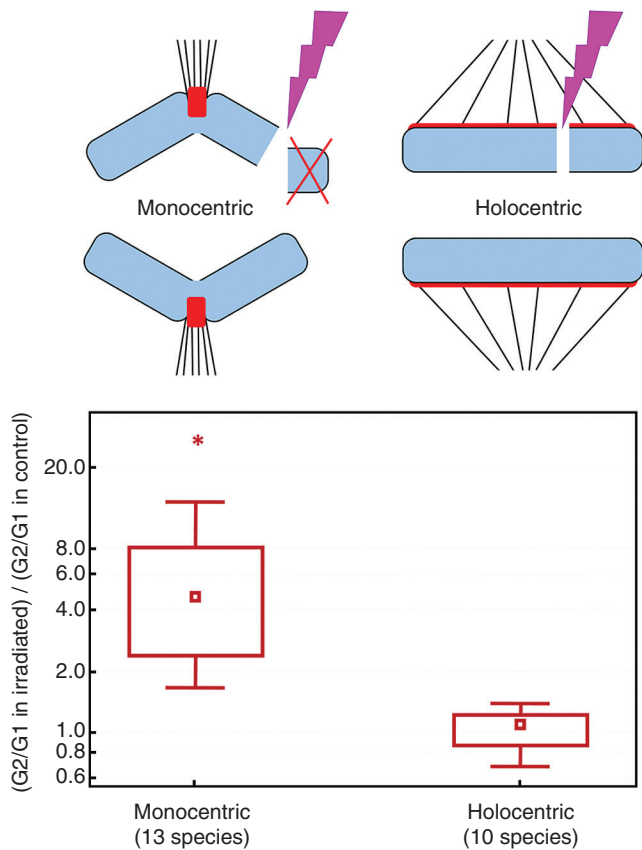


FIG. 1. Fragmentation of holocentric and monocentric chromosomes and gamma radiation response in monocentrics and holocentrics. Top: holocentric chromosomes and monocentric chromosomes are the two alternative chromosomal structures that have evolved in eukaryotes. The reason why holocentric chromosomes tolerate fragmentations is that they attach spindle microtubules along their entire length during cell divisions, and therefore all their fragments are normally inherited by daughter cells that receive a proper set of genetic material. Monocentric chromosomes, by contrast, attach spindle microtubules to the kinetochore (shown in red), which is formed in a small centromeric region, and their fragments without a centromere are distributed randomly to daughter cells and eventually lost, which is often lethal. Bottom: gamma irradiation causes chromosomal fragmentations that need to be repaired, and for that purpose, the cell cycle is arrested in G2 phase in plants. Therefore, the number of G2 cells in gamma-irradiated plants should increase, resulting in a higher G2/G1 ratio. If the G2/G1 ratio of an irradiated plant is divided by the G2/G1 ratio of a non-irradiated control, the resulting value shows the overall response in cell cycle arrest to gamma irradiation (y-axis). These values for 13 monocentric and ten holocentric species are shown in the two box-plots. Relative to monocentrics, there is basically no increase in the G2/G1 ratio in holocentrics after irradiation, suggesting that holocentrics cope with chromosomal fragmentation more effectively. Monocentric species are represented by *Asplenium bulbiferum*, *Begonia bowerae*, *Cymbalaria muralis*, *Euonymus japonicus*, *Kalanchoë delagoensis*, *Lavandula angustifolia*, *Lysimachia nemorum*, *Peperomia glabella*, *Pisum sativum*, *Plectranthus amboinicus*, *Sedum spurium*, *Senecio articulatus* and *Silene nocturna*. Holocentric species are represented by *Carex grayi*, *C. humilis*, *C. pilulifera*, *Drosera capensis*, *D. scorpioides*, *Eleocharis palustris*, *Isolepis prolifera*, *Luzula sylvatica*, *Prionium serratum* and *Scirpus cernuus*. See Zedek *et al.* (2016) for further details.

2013). In contrast, the fragmentation of monocentric chromosomes generates acentric fragments that are randomly distributed to daughter cells and eventually lost in subsequent cell generations (Fig. 1). Moreover, if centric fragments of monocentric chromosomes fuse, they form aberrant dicentric chromosomes (Stear and Roth, 2002; Carpenter *et al.*, 2005; Lowden

et al., 2011). Holocentric chromosomes should therefore provide a selective advantage by directly protecting DNA in times of exposure to agents causing chromosomal fragmentation, i.e. clastogens. Such clastogens may include cosmic radiation (UV, gamma rays, X-rays; Kovalchuk *et al.*, 2000; Waterworth *et al.*, 2011), natural radiation from radioactive elements (Takahashi, 1976), desiccation/freezing (Waterworth *et al.*, 2011) or a broad range of chemicals (Ishidate *et al.*, 1988). However, although holocentric chromosomes tolerate fragmentation, the question as to whether this tolerance also provides a selective advantage over monocentric organisms is an entirely different matter. We are not aware of any systematic research that has investigated the competitiveness of monocentrics and holocentrics or their comparison in clastogenic conditions and considered the potential consequences of holocentric tolerance to fragmentation for the evolution of eukaryotes and holocentrism itself.

Below is a summary of the available evidence that holocentric chromosomes may, indeed, confer a selective advantage in clastogenic environments and conditions. The causes of clastogenic exposure are discussed and it is shown that such conditions, of various duration and intensity, have occurred many times throughout the history of Earth's biota. The role of holocentric chromosomes in eukaryotic evolution is also considered, with a particular emphasis on plant and animal terrestrialization half a billion years ago. The paper then moves on to the negative consequences of holocentrism and discusses potential biases in our knowledge of its distribution across eukaryotes. The paper concludes with proposals for future research that is needed to test the anticlastogenic hypothesis of holocentrism and its evolutionary consequences.

Holocentrics vs. Monocentrics in Clastogenic Conditions

The effects of clastogens have largely been studied regardless of chromosomal structure differences and only rarely were both holocentric and monocentric organisms included in a study. Nevertheless, these studies provide clues suggesting that the possession of holocentric chromosomes may indeed be advantageous in clastogenic environments (see below). Much of the research was conducted during the Cold War period, when interest in understanding the impacts of a nuclear war on life on Earth peaked. In the two-volume book *Environmental Consequences of Nuclear War*, published by the Scientific Committee for Environmental Consequences of Nuclear War, holocentric chromosomes are listed as one of the factors of high radioresistance, while monocentric chromosomes are considered a factor of low radioresistance (Hutchinson *et al.*, 1985).

Studies of the clastogenic effects upon animals that included holocentric species have primarily focused on pest arthropods. Holocentric pest insects and arachnids require, on average, much higher sterilization (Bakri *et al.*, 2005) and disinfestation (Hallman, 2000) doses of gamma or X-ray radiation than monocentrics. Nematodes, which are also holocentric (Fig. 2), are extremely resilient, and quarantine doses for their eradication are approximately one order of magnitude higher than for arthropods (Hallman, 2000).

Plants appear to respond to gamma irradiation via a cell cycle arrest in the G2 phase (Preuss and Britt, 2003;

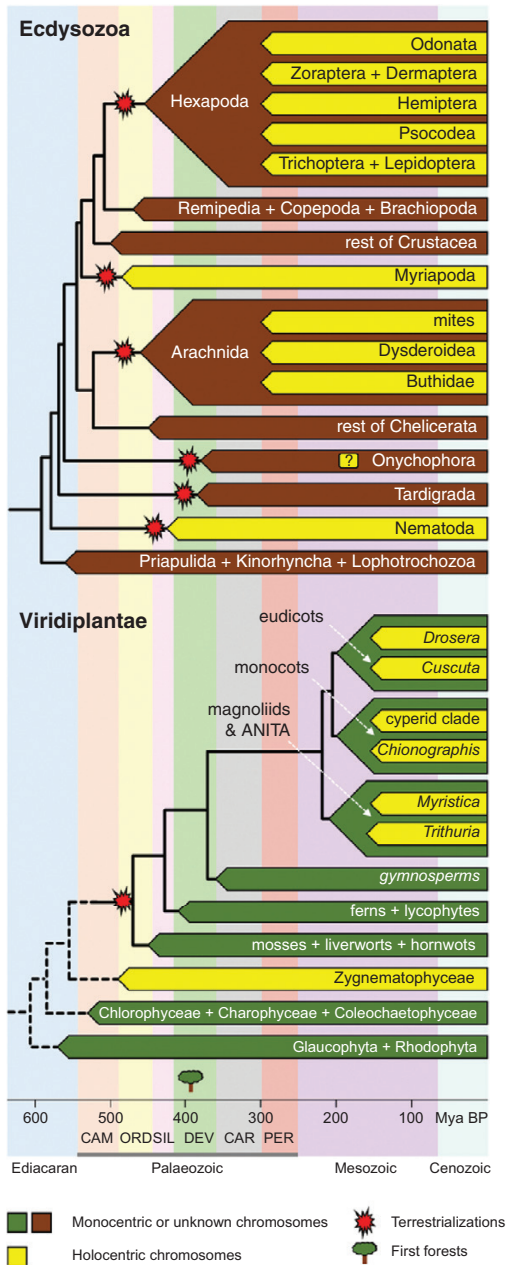


FIG. 2. Phylogenetic distribution of holocentric chromosomes and terrestrialization events. The distribution of holocentric lineages (yellow) and terrestrialization events (red sparks) in eukaryotes is shown on simplified dated phylogenies of Viridiplantae and Ecdysozoa. The remaining lineages, depicted in green in Viridiplantae and brown in Ecdysozoa, are either monocentric or with unknown chromosomal structure, and the ancestral states of these clades can be either monocentric or holocentric (see main text for further discussion). The tree for Ecdysozoa and terrestrialization events was modified from Rota-Stabelli *et al.* (2013). The tree topology and node ages for Viridiplantae are based on Wickett *et al.* (2014) and Kumar *et al.* (2017). Dashed branches in the Viridiplantae tree indicate uncertainty in node ages (not in topology). The cyperid clade in Viridiplantae includes families Cyperaceae, Juncaceae and Thurniaceae.

Culligan *et al.*, 2004). When we gamma-irradiated 13 monocentric and ten holocentric plant species and measured the ratio of G2 cells to G1 cells in newly grown tissues, we (Zedek *et al.*, 2016) found that the ratio increased only in the monocentrics,

suggesting that holocentric plants coped better, or faster, with chromosomal fragmentation (Fig. 1).

Although no studies have intentionally compared the performance of monocentric and holocentric plants under clastogenic conditions in the field, a few have included species with both chromosomal structures. In a 3-year competition experiment in which plants were cultivated in seedbeds along a gradient of gamma radiation, the holocentric species *Juncus tenuis* (Juncaceae) and *Bulbostylis capillaris* (Cyperaceae) dominated in sections that received the highest doses, and their abundance increased along the radiation gradient (McCormick and Platt, 1962). In a seasonally gamma-irradiated forest in northern Wisconsin, the most resistant species were the holocentric *Luzula acuminata* (Juncaceae) and *Carex pensylvanica* (Cyperaceae) (Zavitkovski and Salmonson, 1975). *Luzula acuminata* was also among the four herbs growing closest to the chronic irradiation source in a 14-year experiment conducted in a Canadian boreal forest (Amiro and Dugle, 1985; Amiro and Sheppard, 1994). Two of the three remaining species, *Fragaria virginiana* and *Galium septentrionale*, were monocentric polyploids (Bennett and Leitch, 2012; Rice *et al.*, 2015), which may have strengthened their resistance (Hutchinson *et al.*, 1985). The third species was determined only to the genus level as *Aster* (Amiro and Dugle, 1985). In a long-term experiment conducted for five decades in the pine–oak forest at Brookhaven National Laboratory, *Carex pensylvanica* showed extreme gamma radiation resistance (Stalter and Kincaid, 2009). When the chronic gamma irradiation began in 1962, it created a dead zone of no vegetation (0–20 m from the source), followed by a zone dominated by *C. pensylvanica* (20–40 m from the source) (Woodwell, 1962; Stalter and Kincaid, 2009). *C. pensylvanica* outcompeted other species, and its coverage increased for another 14 years (Flaccus *et al.*, 1974; Olsvig, 1979). After almost 50 years, *C. pensylvanica* still dominates the vegetation zone closest to the source (Stalter and Kincaid, 2009). A similar endurance by a holocentric plant under purely natural conditions can be observed on the Tibetan Plateau, where plants are exposed to intense UV radiation due to high altitude (Willis *et al.*, 2009; Beckmann *et al.*, 2014). One-fifth of the Tibetan Plateau area (approx. 500 000 km²) is covered by mats of the holocentric plant *Kobresia pygmaea* (Cyperaceae) that reach altitudes of almost 6000 m on the slopes of Mt Everest (Miehe *et al.*, 2008) and display coverage ranging mostly between 90 and 98 % (Miehe *et al.*, 2008).

Zygnematophyceae, the only known holocentric class of charophyte algae (King, 1960; Godward, 1966; Mughal and Godward, 1973; Wells and Hoshaw, 1980; Brook, 1981; Godward, 1985), show a remarkable resistance to UV radiation and desiccation (Holzinger and Pichrtová, 2016; Stamenković and Hanelt, 2017). These species are distributed worldwide and dominate in various stressful habitats, such as the surfaces of glaciers (Holzinger and Pichrtová, 2016). Compared to other charophyte algae, Zygnematophyceae are also more resistant to gamma radiation (Vedajanani and Sarma, 1979).

The above examples of experiments and studies on extant species provide evidence that holocentric chromosomes may indeed be advantageous under clastogenic conditions. However, the question remains as to whether, and how often, such conditions that would promote the evolution of holocentric chromosomes occurred over the Earth’s history and the evolution of eukaryotes.

GLOBAL AND COSMIC FACTORS THAT MAY SELECT FOR HOLOCENTRISM

Cosmic radiation, coming mainly from the sun (Dartnell, 2011; Meert *et al.*, 2016), nearby supernovae (Dartnell, 2011) and the interstellar clouds through which our solar system passes (Pavlov *et al.*, 2005), has been accompanying life on Earth throughout its history (Doglioni *et al.*, 2016; Meert *et al.*, 2016). Although the biosphere is protected by the Earth's magnetic field (Pavlov *et al.*, 2005), due to its polarity reversals, this shield is highly unstable over longer evolutionary time scales (Merrill and McFadden, 1999; Hulot *et al.*, 2010; Meert *et al.*, 2016). Such a weakening of the magnetic field can occur more than 20 times per million years (Hulot *et al.*, 2010; Meert *et al.*, 2016), and combined with high-energy cosmic radiation may be a serious threat to Earth's biota (Melott *et al.*, 2004; Pavlov *et al.*, 2005; Wei *et al.*, 2014; Thomas *et al.*, 2015). These events of high-energy radiation influx may last from tens to thousands of years (Meert *et al.*, 2016). Nevertheless, even if global and cosmic conditions are stable, organisms may experience a dramatic exposure to clastogens when they move to a new environment. One of the most important events of this kind occurred when eukaryotes left the oceans to colonize land half a billion years ago.

THE ADVANTAGE OF HOLOCENTRISM DURING EVOLUTIONARY TRANSITIONS

Marine eukaryotes were protected against solar UV radiation by a water column (Doglioni *et al.*, 2016), a protection that is especially effective in coastal waters (Tedetti and Sempéré, 2006). However, conquering the land required the abandonment of this protection and coping not only with more intense radiation but also with the clastogenic effects of desiccation (Waterworth *et al.*, 2011; Lomax, 2012). In this regard, it is of particular interest that the only known holocentric lineage of algae (Zygnematophyceae) is the closest algal relative to land plants, with which they form a monophyletic clade (Fig. 2; Wodniok *et al.*, 2011; Timme *et al.*, 2012; Ruhfel *et al.*, 2014; Wickett *et al.*, 2014). The close relationship of Zygnematophyceae (instead of previously thought Coleochaetophyceae or Charophyceae) and land plants (Wodniok *et al.*, 2011; Timme *et al.*, 2012; Ruhfel *et al.*, 2014; Wickett *et al.*, 2014) has been a surprising finding that is difficult to reconcile with their relatively simple morphology (Delwiche and Cooper, 2015), sparking a debate about zygnematophycean traits and exaptations that may have promoted the colonization of land half a billion years ago (Delwiche and Cooper, 2015; de Vries *et al.*, 2016). We argue that holocentric chromosomes were the exaptation that enabled this lineage to colonize land by directly mitigating the clastogenic effects of cosmic radiation and desiccation on DNA. Moreover, if the life cycle of extant charophyte algae, which is dominated by a haploid phase (Niklas and Kutschera, 2010), is imposed on the algal ancestor of land plants, the importance of holocentrism in plant terrestrialization increases because haploids are more sensitive to radiation (Hutchinson *et al.*, 1985).

While plants successfully colonized land only once, animals succeeded on multiple occasions (Fig. 2; Dunn, 2013; Rota-Stabelli *et al.*, 2013). A recent study dated the first six

independent terrestrialization events in the earliest land animals using both fossil and molecular data (Rota-Stabelli *et al.*, 2013). Ordered based upon the time they invaded land, these lineages were millipedes (Myriapoda), insects (Hexapoda), arachnids (Arachnida), roundworms (Nematoda), water bears (Tardigrada) and velvet worms (Onychophora) (Rota-Stabelli *et al.*, 2013). Because holocentric chromosomes are known from the first four terrestrial lineages (Mola and Papeschi, 2006; Melters *et al.*, 2012; Bureš *et al.*, 2013), where they may represent an ancestral state (Melters *et al.*, 2012; Escudero *et al.*, 2016), holocentrism may have also played an important role in the colonization of land by animals. Holocentrism has been suggested in velvet worms (Mola and Papeschi, 2006), but, thus far, the evidence is weak, and their chromosomal structure needs to be studied in more detail. More detailed analyses are also needed in water bears, which, to date, appear to be monocentric (Bertolani, 1982). It is noteworthy that the invasion of land by water bears and velvet worms occurred in the Devonian, which coincides with the appearance of the first forests and may be associated with this change (Fig. 2; Rota-Stabelli *et al.*, 2013), perhaps because forests provided shade protection against cosmic radiation. The first forests were also involved in the colonization of land by vertebrates (Garwood and Edgecombe, 2011), which are monocentric. In contrast, there was no such protection from the late Cambrian to the early Silurian (Rota-Stabelli *et al.*, 2013) for potentially holocentric colonizers (millipedes, insects, arachnids, roundworms).

Similar to a transition in environmental conditions, a change in nutrition strategy may also create an evolutionary context that selects for holocentric chromosomes. For instance, the transition to herbivory may require an organism to deal with chemical clastogens produced by a host plant (Mandrioli and Manicardi, 2012). Aphids (Hemiptera) that feed on tobacco and lavender exhibit a higher rate of chromosomal fissions due to the clastogenic effects of nicotine (Mandrioli and Manicardi, 2012; Kati *et al.*, 2014) and linalyl acetate (Mandrioli *et al.*, 2014). Accordingly, the majority of phytophagous insect and arachnid species belong to the holocentric orders of true bugs (Hemiptera) and butterflies and moths (Lepidoptera; Wiens *et al.*, 2015), and the holocentric subclass of mites (Acari; Lindquist, 1999; Shultz, 2001), respectively.

ON THE RARITY AND A POTENTIAL DARK SIDE OF HOLOCENTRISM

If holocentrism is so advantageous, why is it so rare among eukaryotes? There are at least four possible explanations for this observation that are not mutually exclusive.

First, the mechanism of the origin of holocentrism from monocentrism may be complex, and thus losing this trait may be much easier than gaining it. Indeed, phylogenetic models have shown that the rate of transition from holocentrism to monocentrism was two orders of magnitude higher than the reverse (Escudero *et al.*, 2016). However, since holocentric chromosomes are basically monocentric chromosomes with a very large kinetochore, this explanation is unlikely. Second, holocentric mutation may not occur frequently enough relative to other anticlastogenic mutations such as polyploidy, which also protects against clastogens (Hutchinson *et al.*, 1985) but

originates much more easily. Third, holocentric lineages may have lower diversification rates relative to monocentric lineages because their tolerance to fragmentation may reduce the potential of clastogenic mutagenesis in speciation. In addition, meiotic holocentrism allows only two crossovers (Nokkala *et al.*, 2004), thereby limiting variation, and therefore the adaptive potential of holocentric lineages. However, the tolerance of holocentric lineages to clastogens may reduce their extinction rate and thus balance the lower speciation rate. In their large-scale analysis of 50 000 species, Escudero *et al.* (2016) found that both speciation and extinction rates of holocentric lineages were lower than those in monocentrics. Nevertheless, the net diversification rate of holocentrics was four times lower relative to that in monocentric lineages (Escudero *et al.*, 2016), suggesting that although holocentric lineages do not go extinct as fast as monocentrics, they speciate much more slowly and are thus less stable on longer evolutionary time scales. However, as the authors themselves state, these results must be viewed as preliminary, mainly due to dataset limitations, and further research on this subject is needed. Fourth, the rare occurrence of holocentric chromosomes may be illusory due to historical and methodical biases (see below).

Holocentric chromosomes, as distinct from monocentric chromosomes, were recognized as late as the mid-1930s (Schrader, 1935). Although the first experimental confirmations of holocentrism followed shortly thereafter (White, 1936; Hughes-Schrader and Ris, 1941), such studies were scarce in karyological literature and thus unable to shake an already strongly enrooted notion that chromosomes are sausage-shaped particles with a localized centromere (i.e. monocentric; see also a historical overview by Battaglia, 2003). Hence, despite the fact that many studies provided only chromosome counts without any further inspection of chromosomal structure, the chromosomes of these investigated taxa often were, and still are, considered monocentric unless proven otherwise (for discussion, see also Wrench *et al.*, 1994; Guerra *et al.*, 2010; Bureš *et al.*, 2013). Even if chromosomal structure is inspected in a given species, there are issues that need to be kept in mind. Chromosomal structure determination cannot be easily applied outside the analysed species. The presence of both monocentric and holocentric species within a genus (*Cuscuta*) or a family (Melanthiaceae) shows that generalizations based on just a few species may be misleading. To arrive at solid conclusions about the evolution of both monocentric and holocentric chromosomes, accurate knowledge of their phylogenetic distribution is needed. Therefore, we advocate for a screening across eukaryotes to search for holocentric chromosomes. There is a broad range of methods for achieving that end based upon either microscopy (e.g. Murakami and Imai, 1974; Gernand *et al.*, 2003; Heckmann *et al.*, 2011; Demidov *et al.*, 2014) or flow cytometry (Zedek *et al.*, 2016).

FUTURE DIRECTIONS

Although holocentric chromosomes may be advantageous in clastogenic environments (see above), holocentrism is not the only way of coping with clastogens. Other ways may include, but are not limited to, changes in genomic parameters, physiology and reproduction, behavioural adaptations or protective

pigment production (Hutchinson *et al.*, 1985; Willis *et al.*, 2009). Similarly, holocentrism may be an adaptation to another factor, such as centromere drive (see above). Therefore, the hypothesis of holocentrism as an anticlastogenic adaptation must be systematically tested. Such testing can be done from several perspectives including controlled laboratory experiments, field studies across clastogenic gradients and large-scale phylogenetic analyses.

Controlled laboratory or field experiments should focus on related taxa that differ in chromosomal structure but are similar in other aspects of their morphology, ecology or life history and should compare their performance and competitive abilities under clastogenic conditions. In plants, such pairs can be monocot families such as Cyperaceae and Poaceae that are distributed worldwide and share many important traits and characteristics (Bouchenak-Khelladi *et al.*, 2014). However, the former family is holocentric, while the latter family is monocentric. Other promising candidates may be closely related monocentric and holocentric species from the parasitic genus *Cuscuta* (Convolvulaceae) or the carnivorous family Droseraceae. In animals, similar comparisons could be made using species from monocentric and holocentric orders of arthropods. Cell and callus cultures that have been established from holocentric and monocentric insect (Smagge *et al.*, 2009) and plant species (Madej and Kuta, 2001; Banasiuk *et al.*, 2012) may also be useful, especially for treatments with chemical clastogens. Because chemicals, including clastogens, are part of plant defence against pests, holocentric herbivorous insects could display a broader host range and/or feed more often on plants displaying higher concentrations of clastogenic compounds.

UV radiation varies with latitude and increases with altitude (Willis *et al.*, 2009; Beckmann *et al.*, 2014), providing clastogenic gradients along which the performance and competitive abilities of monocentrics and holocentrics can be studied in nature. Along a given gradient, holocentric species should display a weaker response to increasing UV intensity than related monocentric species. The proxy for such a response could be the proportion of endopolyploid cells (Gegas *et al.*, 2014) or the concentration of UV-absorbing compounds (Willis *et al.*, 2009; Newsham and Davidson, 2012). The European Vegetation Archive, a recently released database of more than a million vegetation plots (Chytrý *et al.*, 2016), is a unique source of data that can be used in combination with UV radiation gradients. We would expect the data to show an increase in the relative abundance and coverage of holocentric species in vegetation plots exposed to more intense UV radiation.

Reversals of the magnetic field polarity (Merrill and McFadden, 1999; Hulot *et al.*, 2010), fluctuations in solar activity (Dartnell, 2011; Thomas *et al.*, 2015), supernovae (Melott *et al.*, 2004; Dartnell, 2011) and the passage of the Earth through interstellar clouds (Pavlov *et al.*, 2005), alone or combined, have exposed Earth's biota to a frequent bombardment by cosmic radiation throughout its history (Hulot *et al.*, 2010; Wei *et al.*, 2014; Doglioni *et al.*, 2016; Meert *et al.*, 2016) and provided many opportunities for utilization of holocentric chromosomes. The reversals of the magnetic field polarity, for instance, are dated back 600 Myr (Merrill and McFadden, 1999; Hulot *et al.*, 2010; Meert *et al.*, 2016). Since large dated phylogenies of eukaryotes, including plants, are also available (e.g. Zanne *et al.*, 2014; Kumar *et al.*, 2017), it should be possible

to test whether the evolution of holocentric lineages coincides with the periods of frequent influx of intense cosmic radiation and other potentially clastogenic events over Earth's history.

CONCLUSIONS

The tolerance of holocentric chromosomes to fragmentation implies that the possession of holocentric chromosomes may confer an advantage in clastogenic conditions and environments. Although this possibility has never been directly studied, it can be deduced from several studies that have included and compared the performance of holocentric and monocentric organisms. Events of acute or chronic exposure to clastogens of varying intensities and duration have been accompanying life on Earth throughout its history, possibly explaining the repeated origin of holocentric chromosomes in eukaryotes. One such event occurred half a billion years ago, when eukaryotes left the oceans and colonized the land, thereby losing their water-column shield against the clastogenic effects of cosmic radiation and desiccation. There is also evidence that the first terrestrial lineages of plants and animals may have been holocentric. The anticlastogenic hypothesis of holocentrism can be tested on several scales and different perspectives from laboratory experiments through field studies to large phylogenetic comparative analyses. We conclude that the far-reaching consequences of holocentrism for eukaryotic evolution is a possibility worth pursuing in future research and that holocentric chromosomes should no longer be viewed as a peripheral peculiarity.

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