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Source: Systematic Botany, 41(3):580-585.
Published By: The American Society of Plant Taxonomists
URL: http://www.bioone.org/doi/full/10.1600/036364416X692442

Systematic Botany (2016), 41(3): pp. 580–585

DOI 10.1600/036364416X692442
Date of publication August 26, 2016

The Phylogenetic Origins and Evolutionary History of Holocentric Chromosomes

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Communicating Editor: James Smith

Abstract—In eukaryotes, we can recognize two kinds of chromosomes, based on the location of the kinetochores. The majority of eukaryotes have monocentric chromosomes, in which kinetochoric activity is concentrated in a single locus. In several unrelated eukaryotic lineages, chromosomes are holocentric, having diffuse centromeric / kinetochoric activity along the length of the chromosome. Whether holocentric chromosomes are derived or ancestral is still under debate. This study uses the phylogenetic tree from Time Tree of Life project, comprising more than 50,000 sampled species, to reconstruct the evolution of holocentry. Asymmetrical two-state Markov (Mk2) models were compared with BiSSE models to assess sensitivity of our conclusions to possible effects of holocentry on lineage diversification rates. Our analyses based on Mk2 and BiSSE models inferred that the rate of transition from holocentric to monocentric chromosomes is two orders of magnitude higher than the reverse direction. The ancestral state of all eukaryotes is ambiguous depending on the model, inferred to be either monocentric (Mk2) or holocentric (BiSSE). Whatever the direction, the multiple transitions and high diversity of centromere organization across the tree of life are what we would expect if there are selective advantages to both chromosome types. Understanding those selective advantages is key to understanding how genetic information is organized and transmitted from one generation to the next, and why these major evolutionary transitions in centromere organization have occurred in the first place.

Keywords—BiSSE, centromere, holokinetic, kinetochore, Markov, monocentric chromosomes

The centromere or kinetochore is the point of attachment of kinetochore proteins to the chromosomes. During cell division, spindle fibers attach to the kinetochores to pull the chromosome to one pole or the other. In eukaryotes, we recognize two kinds of chromosomes, based on the location of the kinetochores. The majority of the eukaryotes have monocentric or monokinetic chromosomes, in which the kinetochoric activity is concentrated in a single locus, the centromere. In a minority of eukaryotes from several lineages, chromosomes are holocentric or holokinetic, with centromeric activity distributed along the length of the chromosome (Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013).

In organisms with monocentric chromosomes, chromosome fragments without centromeres are not able to segregate to their corresponding pole during meiosis. Thus chromosome fission will usually result in a loss of genetic material, which we often expect to produce unviable or low-fitness gametes. However, in organisms with holocentric chromosomes, diffuse kinetochoric activity ensures that even chromosome fragments segregate during meiosis. Holocentry thus has the potential to render chromosome fissions essentially neutral, and to increase the rate of evolution of fusions and translocation (Mola and Papeschi 2006; Hipp et al. 2009; Hipp et al. 2013).

The most studied organism with holocentric chromosomes is the nematode and model organism *Caenorhabditis elegans*. However, chromosomes in *C. elegans* and many hemipterans behave functionally as monocentric organisms during meiotic divisions (Dernburg 2001) so that their rate of fixation of chromosome rearrangements may be reduced relative to holocentric organisms that do not behave like monocentrics. In fact, in a genome mapping study (d'Alençon et al. 2010), the rate of chromosome rearrangements in the holocentric clade Lepidoptera (butterflies and moths) was higher than that in nematodes, and nematode chromosome rearrangements were approximately four times as rapid as in the monocentric *Drosophila* (fruit flies). There are really only two holocentric groups that show extraordinary chromosome number variation: the insect order Lepidoptera, in which the families Lycaenidae (*Agrodiaetus* butterflies, $2n$ = 20–268; Lukhtanov et al. 2005; Kandul et al. 2007) and Nymphalidae (tribe Ithomiini, $2n$ = 10–240; Dinca et al. 2011) show extensive chromosome differentiation; and sedges, the angiosperm family Cyperaceae ($2n$ = 4–226) and especially the genus *Carex* ($2n$ = 12–124; Roalson 2008).

Holocentry has several implications for chromosome evolution, and potential implications for lineage diversification and speciation. In Lepidoptera, chromosome evolution is believed to play a role in reinforcing speciation (Lukhtanov et al. 2005, 2011; Dinca et al. 2011; Kandul et al. 2007). In *Carex*, chromosome differentiation has been demonstrated to correlate with genetic divergence within species (Escudero et al. 2010), among populations within species (Hipp et al. 2010), and within populations (Escudero et al. 2013a). These studies suggest that chromosome rearrangements contribute to genetic differentiation at different evolutionary scales in at least *Carex* and Lepidoptera. Beyond lineage diversification and speciation, chromosome number may be an important determinant of recombination rates in organisms with holocentric chromosomes, as has been demonstrated in achiasmatic male meiosis in bed bug (Hemiptera; Bigliardo et al. 2011; Nokkala et al. 2004) and suggested in *Carex* (Escudero et al. 2012; Escudero et al. 2013b). Holocentry appears to have also contributedto the evolution of the sex-determining system in the true bugs. Alternative sex chromosome mechanisms have evolved by fission and fusion. (Hemiptera; Viera et al. 2009).

Holocentric chromosomes are found in several other unrelated lineages in eukaryotes. One of the most recent reviews of holocentry (Melters et al. 2012) reports 768 holocentric eukaryotic species, including 472 insects, 228 plants, 50 arachnids, and 18 nematodes. Lineages in eukaryotes with holocentric chromosomes are: (1) the angiosperm clades *Drosera* (Droseraceae), *Cuscuta* (Convolvulaceae), Melanthiaceae, and the clade formed by families Cyperaceae and Juncaceae;

(2) nematode orders Rhaditida and Tylenchida and the family Ascarididae; (3) insect orders Ephemeroptera, Odonata, Zoraptera, Dermaptera, Psocoptera, Phthiraptera, Thysanoptera, Hemiptera, Trichoptera and Lepidoptera; (4) centipede orders Lithobiomorpha and Scutigeromorpha; (5) scorpions of the superfamily Buthoidea; (6) mites and ticks of the superorder Acariformes and genus *Rhipicephalus* (Ixodidae); (7) microwhip scorpions of order Palpigradi; and (8) six-eyed spiders of families Dysderidae and Segestridae. This review also highlights several cases in which reports of holocentric chromosomes have been later corrected by other studies: the moss *Pleurozium schreberi* and the marine alga *Spirogyra* (Zygnemataceae), and perhaps *Zygnema* from the same family and *Cosmarium* and *Pleurotaenium* (Desmidiaceae) (Godward 1954; Vaarama 1954; Mughal and Godward 1973; Kuta et al. 1998, 2000). Several additional clades have been reported as having holocentric chromosomes but were not included in Melters et al.'s (2012) review: (9) the flowering plant groups *Myristica fragans* (Myristicaceae) and families Cannaceae, Musaceae, Heliconiaceae, Zingiberaceae and Strelitziaceae in the order Zingiberales; (10) the velvet worm *Peripatus* (Peripatidae); and (11) several species from superclade or kingdom Rhizaria such as *Aulacantha scolymantha* from family Aulacanthidae (order Phaeocystina) and *Spongospora*, *Sorosphaera* and *Plasmidiophora* from family Plasmidiophoraceae (order Plasmidiophorales) (Mola and Papeschi 2006; Hipp et al. 2013). Nevertheless, in the case of *Myristica fragrans*, the lack of detailed cytological analysis suggests that the presence of holocentric chromosomes is still uncertain (Bureš et al. 2013). Whether holocentric chromosomes are derived or ancestral in eukaryotes is still under debate (reviewed in Mola and Papeschi 2006). Melters et al.'s (2012) study based on a phylogenetic reconstruction reported that holocentric chromosomes are derived and originated 13 times independently (four in plants and nine in animals).

The aim of this study is to disentangle the mode and tempo of evolution of holocentric chromosomes. Specific questions are (i) Are holocentric chromosomes ancestral or derived within eukaryotes? (ii) When and in what lineages did holocentry and monocentry arise in the evolution of eukaryotes? and (iii) How often and in what lineages have there been reversals in chromosome structure?

Materials and Methods

The Phylogenetic Tree and the Trait—The recently published phylogenetic tree by the Time Tree of Life project (http://www.timetree.org/; Hedges et al. 2015) was used for the reconstruction analyses. This phylogeny contains more than 50,000 species (most of them eukaryotes) and information of absolute times of diversification. Prokaryote species were pruned from the tree using the function drop.tip as implemented in the R package ape (Paradis et al. 2004; R Core Team 2015).

Only a small minority of eukaryotic species have actually been studied cytogenetically, with an eye to distinguishing between holocentry and monocentry. There is a large quantity of information about chromosome evolution in several clades, for example the family Cyperaceae (Roalson 2008), but little information in other clades of the tree of life (e.g. the super clade Rhizaria). We therefore had to make several assumptions in coding chromosome character states on the phylogeny. All eukaryote species were assumed to have monocentric chromosomes unless a scientific publication could be found demonstrating or persuasively arguing for holocentry. With the objective of avoiding false positives, we only coded as having holocentric chromosomes the clades without doubts (although we do not rule out having false positives in our data set). This introduces a potential bias: our character state coding presumably underestimates the frequency of holocentry. Thus conclusions drawn in this study will need to be evaluated in light of any future findings of new holocentric lineages.

On the other hand, even when a clade (i.e. family) has been reported as having holocentric chromosomes, sampling is typically too sparse to allow us to detect reversions to monocentry. For example, although there is vast information about holocentric chromosomes in some plant families like Cyperaceae (ca. 5,500 species; Roalson 2008) and no species in this family have ever been reported as not having holocentric chromosomes, we could not reject the possibility that some of the over 5,500 species in this family could have monocentric chromosomes, as only approximately 10% of species in the family have been investigated. The same is true of the sister family Juncaceae (the rushes), in which every species investigated to date has been shown to be holocentric. In fact, holocentry has been described as the "one obvious and convincing chromosomal higher-level synapomorphy in monocotyledons," referring to the Cyperaceae + Juncaceae clade (Greilhuber 1995: 380). It appears that there is only one plant family with both kinds of chromosomes: the family Convolvulaceae exhibits holocentry in only some clades of genus *Cuscuta*. The same is true in butterflies and moths, in which the entire order (Lepidoptera) is widely assumed to be holocentric based on the broad comparative cytogenetic work that has been done to date (see Melters et al. 2012). Based on this pattern of large-scale holocentry, we treat higher-level clades primarily in this study, except where evidence allows us to more finely divide lineages. As a consequence, our inferences in this study will largely ignore dynamics within larger clades, as the weight of evidence suggests that transitions within families are rare indeed.

All higher holocentric clades were present in the Time Tree of Life phylogeny except the insect order Zoraptera, the lineages of algae, and moss (but there are doubts about these two; see above), and the lineages in the superclade Rhizaria. Those lineages are excluded from analyses presented here. All data (trees, data sets and codes) are available from the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.405g1.

Models of Evolution—We reconstructed the evolution of holocentric (h) vs. monocentric (m) chromosomes as a binary character using the two-state asymmetrical Markov model (Mk2) as implemented in the R package diversitree (FitzJohn 2012). The Mk2 model has one parameter for the rate of transition from monocentric to holocentric chromosomes (q_{mh}) and another (q_{hm}) for rate of transition from holocentry to monocentry. Reconstruction of binary characters using the Mk2 model is known to be biased when the effect of the character state on rates of speciation and extinction is neglected (Goldberg and Igic 2008); in such cases, the Mk2 model may fail to detect genuinely irreversible evolution, and effects of a trait on diversification may be mistakenly interpreted as a rate of trait transition (Maddison 2006). We repeated the reconstruction using the BiSSE model, which jointly models rates of trait evolution and the effects of trait states on lineage diversification (Maddison et al. 2007). The BiSSE model incorporates four additional parameters beyond the rates of character state transitions: the rate of speciation for each character state (λ_m and λ_h) and the rate of extinction for each character state (μ_m and μ_h). We accounted for incomplete taxon sampling in BiSSE inferences by assuming random sampling from the tree of life, using several estimates of extant eukaryote species diversity (2E06, 5E06 and 8E06 extant species; Costello et al. 2013). The results from analyses using BiSSE on a subset of taxa must be interpreted with caution, as parameter estimates under the BiSSE method for incompletely sampled phylogenies may not be accurate or precise below ca. 50% sampling (FitzJohn et al. 2009; FitzJohn 2012). Under our lowest estimate of species diversity (2E06 spp.), our sample is approximately 2.5% of the total eukaryotic diversity. In addition, we model missing species as though they were a random sample of eukaryote species, drawn equally from all lineages of the tree and in both character states, which is probably not the case.

For computational reasons (our phylogeny had over 50,000 tips) all analyses were performed using maximum likelihood.

Although we have used a binary character in our analyses (monocetric vs. holocentric), we are aware that there are more atypical centromeres which could constitute intermediate states between holocentric and monocentric (such as de novo formed centromeres and terminal neocentromeres as well as di-, tri- and metapolycentromeres; Cuacos et al. 2015). These kind of atypical centromeres are more unstable, rarer and little-known. Including such rarities in our analyses would likely do little to clarify the evolutionary history of holocentry.

Results

Our phylogenetic tree, after pruning the prokaryote species, had 50,455 eukaryote tips. The inferred parameters of

the Mk2 model were $q_{mh} = 9.155 \times 10^{-6}$ and $q_{hm} = 1.068 \times 10^{-4}$ which suggest that the rate of transition from holocentry to monocentry is two orders of magnitude higher than the reverse rate. The constrained single-rate models (number of parameters = k = 1, $q_{mh} = q_{hm}$, AIC = 508.13; k = 1, $q_{mh} = 0$, AIC = 951.35) had much less support than the unconstrained 2-rate model (k = 2, AIC = 456.70). The constrained model with the root fixed as holocentric (k = 2, AIC = 470) had little support in comparison with the unconstrained model (k = 2, AIC = 456.70). The unconstrained Mk2 reconstruction implies that holocentry derived from monocentry in 19 independent origins (Fig. 1). Holocentry under this model arises in six plant lineages (Droseraceae, *Cuscuta*, Myristicaceae, Melanthiaceae, Zingiberales, and Juncaceae + Cyperaceae), one lineage in nematodes, one lineage in velvet worms, five insect lineages independently (Odonata, Dermaptera, Psocoptera + Phthiraptera + Thysanoptera + Hemiptera -PPTH-, Lepidoptera + Trichoptera and Ephemeroptera), two centipede lineages independently (Scutigeromorpha and Lithobiomorpha) and four independent lineages in arachnids (the lineage including all arachnids and three nested lineages: then one lineage of mites and ticks, one lineage superfamily Buthoidea and one lineage of six-eyed spiders). We have also inferred ten reversions from holocentry to monocentry (Fig. 1): three in Zingiberales (families Lowiaceae, Marantaceae and Costaceae), one in nematodes (family Anisakidae), and six in arachnids (1. Opiliones, 2. Mesostigmata + Ixodida + Opilioacarida, 3. Solifugae + Ricinulei, 4. Uropygi + Scorpiones + Amblypygi + Araneae, 5. Xiphosura and 6. Pseudoscorpiones).

The inferred parameters of the BiSSE model assuming a total of 5E06 extant species were $q_{mh} = 8 \times 10^{-6}$ and $q_{hm} = 7.54 \times 10^{-4}$, again suggesting that the rate of transition from holocentry to monocentry is two orders of magnitude higher than from monocentry to holocentry. The rate of speciation associated with the monocentric state ($\lambda_m = 7.768$ lineages per My (lin. My^{-1})) was higher than for the holocentric state ($\lambda_h = 5.703$ lin. My^{-1}). The rate of extinction associated with the monocentric state ($\lambda_m = 7.759$ lin. My^{-1}) was also higher than for the holocentric state ($\lambda_h = 5.701$ lin. My^{-1}). Nevertheless, the net diversification rates were hardly different ($r_m = 0.0090$ lin. My^{-1}, $r_h = 0.0019$ lin. My^{-1}). This is expected given that the rates of trait evolution are the same in the BiSSE and Mk2 models; only when trait state has an effect on diversification rate would we expect BiSSE and Mk2 models to imply different rates of trait evolution. Constrained models (k = 5, $\lambda_{mh} = \lambda_{hm}$, AIC = 428200; k = 5, $\mu_{mh} = \mu_{hm}$, AIC = 428204) had little support in comparison with the full model (k = 6, AIC = 428146). When assuming that the species richness in eukaryotes are 2E06 or 8E06, we obtained very similar parameter estimates, with minor differences in the speciation and extinction rates (λ_{mh}, λ_{hm}, μ_{mh} and μ_{hm}) depending on how many extant species we considered in the eukaryotes.

Character state reconstruction using BiSSE (species richness 5E06) suggests that monocentry is derived from holocentry. The constrained model with the root fixed as monocentric (k = 6, AIC = 428172) had little support in comparison with the unconstrained model (k = 6, AIC = 428146). The unconstrained BISSE reconstruction implies that monocentry derived from holocentry in 171 independent origins, specifically in 62 nodes and 109 terminals (Supplementary Fig. 1, available from the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.405g1). We also inferred eight independent reversions from monocentry to holocentry

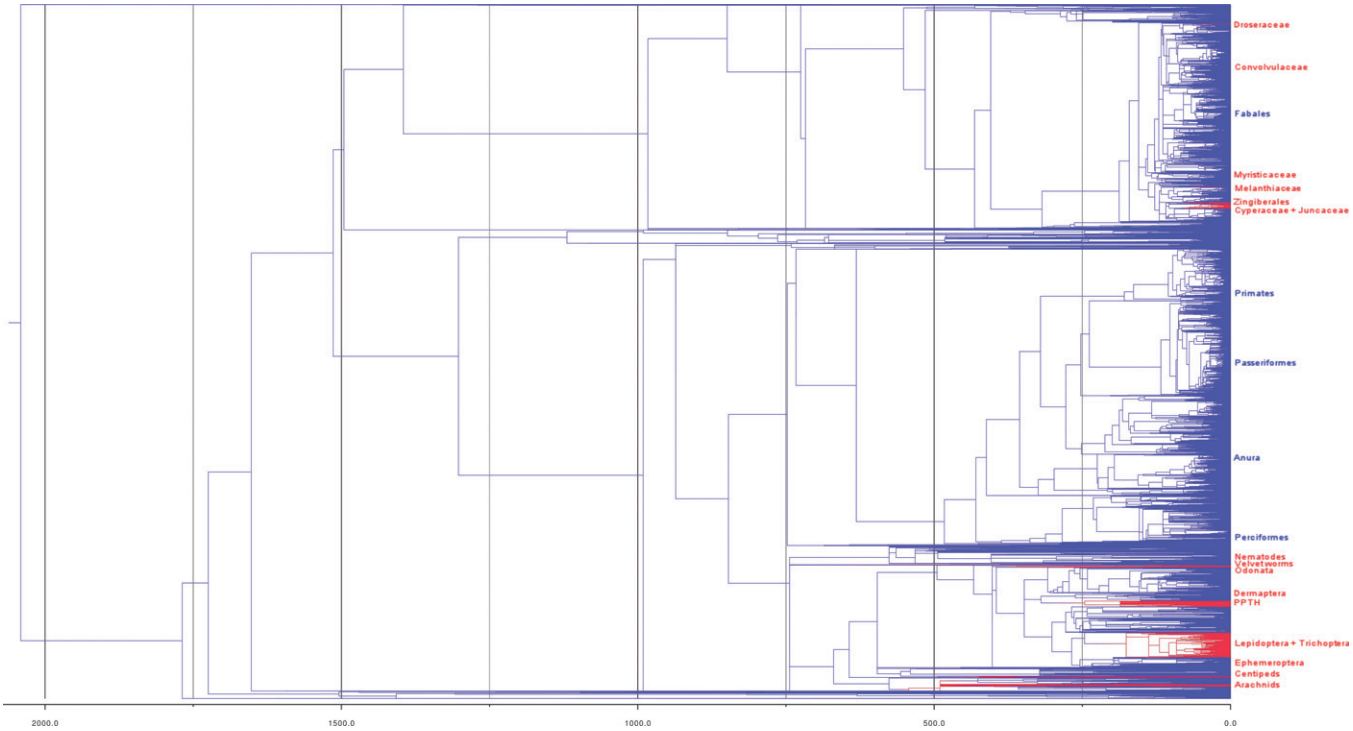

FIG. 1. Phylogenetic tree of eukaryotes with 50,455 species derived from the Time Tree of Life project. The ancestral state reconstruction of monocentric vs. holocentric chromosomes based on the Mk2 model is mapped on the branches using colors (blue for monocentric and red for holocentric). All groups with holocentric chromosomes are labeled in the phylogeny in red. Some representative groups of organisms with monocentric chromosomes are also indicated in blue. The scale axis is in millions of years.

in six plant lineages (Droseraceae, *Cuscuta*, Myristicaceae, Melanthiaceae, Zingiberales and Juncaceae + Cyperaceae), one lineage of insects (Trichoptera + Lepidoptera), and a lineage including arachnids (6-eyed spiders).

Discussion

The Dominant Hypothesis: Holocentric Chromosomes Are Derived from a Monocentric Ancestor—The majority of authors on the subject (e.g. Swanson 1957; Greilhuber 1993; Dernburg 2001) have hypothesized that holocentry is the derived chromosomal structure based on the evidence that holocentric chromosomes characterize a relatively small number of eukaryotic lineages. Greilhuber (1993) proposed that holocentry may have arisen as result of the expansion of kinetic activity from a single original location by means of transposons. Nagaki et al. (2005) proposed that holocentry could have been acquired independently during plant and animal evolution by a different mechanism: they hypothesized that if the direction of formation of kinetochores turns by 90° and occurs along the chromosome axes up to the telomeric regions, it could generate holocentric chromosomes. A third possible mechanism was proposed by Villasante et al. (2007a, b), who suggested that centromeres were derived from telomeres during the evolution of the eukaryotic chromosome. The breakage of the ancestral circular genome, in their scenario, prompted the transposition of retroelements and the formation of telomeres, and subtelomeric repeats were the origin of the first centromere. During the transition from actin-based genophore partition to a tubulin-based mechanism of chromosome segregation, pseudodicentric chromosomes increased the tendency toward chromosomal breakage and instability. A continuous spreading of end sequences throughout the chromosome could explain a monocentric to holocentric transition (Villasante et al. 2007a, b). Malik and Henikoff (2009) suggested that holocentric chromosomes evolved as an adaptation to suppress centromere drive by preventing the accumulation of a contiguous block of centromeric satellites. Interestingly, a new model of centromere drive for holocentric organisms, the holokinetic drive, has been proposed to explain the size and complexity of holocentric choromosome evolution based on preferential segregation dependent on chromosome size (Bureš and Zedek 2014).

Our results based on the Mk2 model, which inferred a rate of transition two orders of magnitude higher from holocentric to monocentric than the opposite, support this widely accepted hypothesis. In our Mk2 reconstruction, holocentry arose 19 times in the eukaryotic tree of life, six times in plants and 13 times in animals (once each in nematodes and velvet worms, five times in insects, twice in centipedes, and four times in arachnids). Interestingly, these results suggest that the evolution from monocentric to holocentric chromosome occurred much deeper in animals than in plants (Fig. 1). Our results are congruent with a parsimony-based phylogenetic comparative analysis (Melters et al. 2012) that inferred 13 independent origins (nine in animals and four in plants). While the most parsimonious reconstruction (Melters et al. 2012) recovered a holocentric ancestor of all insects and several reversions to monocentry, our Mk2 results suggest five independent origins of holocentry in insects and no reversions. Nevertheless, Drinnenberg et al. (2014) looked at the presence of CENP-A/cenH3 which is the centromeric specific histone H3 variant in insects and they found that holocentric insects lack CENP-A. Drinnenberg et al. (2014) also reconstructed the phylogeny of CENP-A and inferred that CENP-A was lost independently four times. That study argues against a single transition from monocentry to holocentry followed by several reversals to monocentry, which supports our findings. Finally, Melters et al. (2012) also has more limited tip sampling and did not consider velvet worms and several families in the order Zingiberales and family Myristicaceae as having holocentric chromosomes (Mola and Papeschi 2006; but see previous concerns about *Myristica fragans* having holocentric chromosomes, Bureš et al. 2013).

Our analysis also detected ten reversions to monocentry: three in Zingiberales, one in nematodes and six in arachnids. Further observational studies, however, may demonstrate that some of those reversions are just an incorrect coding of holocentric condition. For example, additional lineages within the angiosperm order Zingiberales or the nematode family Anisakae may have holocentric chromosomes. Melters et al. (2012) did not infer any of these inferred reversions to monocentry, which may be explained by the limited sampling of lineages in their phylogeny.

It is worth noting that our inference of reversions (from an unorganized diffuse kinetochore to an organized centromere) in what might be thought an irreversible loss of complexity (from an organized centromere to a diffuse kinetochore) may be artefacts of using the Mk2 model to reconstruct ancestral states (Goldberg and Igić 2008). However, the facts that our ancestral state in this reconstruction is monocentric and that the BiSSE analysis also shows reversions both support this not being merely artefactual. It appears from this analysis that the loss of monocentry is not irreversible.

The Jury Is Still Out: Might Holocentric Chromosomes Still Be Ancestral?—Holocentric chromosomes have been proposed by a smaller number of researchers to be ancestral to monocentric chromosomes. Localized centromeres have generally been considered to be a more specialized structure than diffuse kinetochores (Schrader 1947; Sybenga 1981), which argues for evolution from holocentry to monocentry. Moreover, in the plant families Amaryllidaceae and Poaceae, holokinetic behavior has been sometimes found in species that generally behave as monokinetic (Bajer 1968; Rhoades 1952), which has been interpreted as evidence that the structure of the ancestral genome resembles a holocentric chromosome (Moore and Aragón-Alcaide 1997). Third, holocentric structures from different unrelated lineages display a very similar structure: a long kinetochore plate at the external side of each sister chromatid (Moore et al. 1999; Nagaki et al. 2005; Guerra et al. 2010). Sybenga (1981) proposed a gradual transition from fully holokinetic to monokinetic chromosomes. The main advantage of localized centromeres is that the concentration of kinetic activity in a single location in each chromosome, separate from chiasmata (cross-over events), facilitates segregation of all chromosomes (Egel and Penny 2008). Holocentric chromosomes typically have only one or two chiasmata during meiosis (Bigliardo et al. 2011; Escudero et al. 2012) and they fail to segregate correctly when there are three or more chiasmata (Nokkala et al. 2004). Monocentry may thus allow a higher rate of recombination within chromosomes; this is compensated for in part by higher chromosome number variability in many holocentric organisms (e.g. Escudero et al. 2012).

Our results based on the BiSSE model imply a holocentric ancestor to all eukaryotes. We view this result with skepticism based on our very low sampling (< 3% of species).

Even with correction for missing taxa, BiSSE parameter estimates have been shown to be fairly robust only to 50% missing taxa. The BiSSE transition rate parameter estimates are similar to those of the Mk2 model, and net diversification rate for holocentric lineages differs little if at all from net diversification rates for monocentric lineages. However, the BiSSE model suggests 171 independent origins of monocentry followed by eight reversions to holocentry. Given the limitations of our dataset in terms of sampling, but also the potential biases in the Mk2 model (Goldberg and Igić 2008), we view our phylogenetic analyses presented here as preliminary, a first step toward addressing the evolutionary history of holocentry.

All analyses presented here, however, support the inference that there have been fundamental transitions or major evolutionary transitions (*sensu* Maynard Smith and Szathmary 1995) in the way that genetic information is organized and transmitted from one generation to the next. A direction of chromosome evolution from holocentry to monocentry still strikes us as unlikely given the relative minority of eukaryotic lineages that are holocentric. However, whatever the direction, the multiple transitions and high diversity of centromere organization across the tree of life are what we would expect if there are selective advantages to both chromosome types. Understanding the selective advantages and the evolutionary history of holocentry is, in our view, one of the keys to understanding how genetic information is organized and transmitted from one generation to the next, and why these major evolutionary transitions in centromere organization have occurred in the first place.

Acknowledgments. We would like to thank James Smith for his help in organizing this proceedings, and American Society of Plant Taxonomists and the Botany 2015 organizing committee for supporting the symposium in which this paper was presented: "Ecological diversification and niche evolution in the temperate zone's largest genus: Carex." We are also grateful to two anonymous reviewers for their comments that greatly contributed to the quality of this manuscript. Funding for this work was provided by the National Science Foundation (Award #1255901 to ALH).

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