**Title:** Not all centromeres are equal, or are they?

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**Abstract**

Despite a century of research, many fundamental aspects of chromosome number evolution remain a mystery. One example is the dynamics of fissions and fusions in holocentric and monocentric chromosomes. Holocentric chromosomes have centromeres that are diffuse and spindle fibers attach along the entire length of the chromosome, while monocentric chromosomes have a single, localized centromere. This difference in arrangement has led to the hypothesis that species with holocentric chromosomes can tolerate higher rates of fusions and fissions as compared to monocentric chromosomes, which may generate chromosomal fragments lacking centromeres that cannot segregate in monocentric species. To test for differences in the rates of fusions and fissions, we analyzed data from 12,412 species of insects in both a taxonomic and phylogenetic framework. Insects show a wide distribution of both type and number of chromosomes within their genomes and provide a good system to study chromosome number evolution. We found that species with holocentric chromosomes had similar rates of chromosome evolution to species with monocentric chromosomes. When separated by order, we found that there were no significant differences between orders in rates of chromosome evolution when polyploidy was included. However, when polyploidy was excluded from our model, Lepidoptera had an increase in the rate of fissions and fusions, but the overall rates for chromosome evolution were not different between holocentric and monocentric clades.

**Introduction**

Chromosome number stability is expected among lineages as shifts in chromosome number can lead to a decrease in fitness. This stability in chromosome number is driven by underdominance of chromosomal rearrangements that cause speciation by reducing the fitness of heterozygotes and suppressed recombination when chromosomal rearrangements are neutral (Faria and Navarro 2010). The evolution of chromosome number has been recalcitrant to the formation of rules or generalizations that can explain variation in patterns and rates across large clades. However, within clades, fusions and fissions are two of the dominant forces in reshaping karyotypes (Lucek 2018). We use these terms for simplicity to describe single chromosome number changes. However, in reality fusions decreasing chromosome number capture two different processes at the molecular level. First, Robertsonian translocations followed by the loss of the short arms can decrease chromosome number. Second, fusion of telomeres from two chromosomes followed by inactivation of one of the centromeres can occur and has occurred in chromosome 2 in humans (Miga 2017). In contrast, fissions increasing chromosome number can occur through fissions in the centromere region and the gaining of new telomeric sequences (Moretti and Sabato 1984; Garagna, et al. 1995).

Although fissions and fusions occur frequently due to errors in recombination, chromosome number is stable amongst clades. The stability of chromosome number is produced by heterozygote disadvantage associated with chromosomal rearrangements and as such these rearrangements should only fix in a population with low effective population size. However, centromeric structure may modulate the fitness effect of fusions and fissions. Since holocentric centromeres are diffuse and spindle fibers attach along the entire length of the chromosome, species with holocentric chromosomes should have little difficulty segregating chromosomes that have experienced fusions or fissions (Malheiros-Garde and Gardé 1950; Greilhuber 1995; Luceño and Guerra 1996). Single chromosome fusion and fission events in holocentric chromosomes do not appear to be underdominant and fragments created during fissions of these chromosomes have been observed to segregate normally during meiosis (Faulkner 1972; Cope 1985). Therefore, holocentricity has the potential to reduce or eliminate selective pressure against and underdominance of chromosomal rearrangements. This could allow for a higher rate of fixation (Escudero, et al. 2012). Despite this prediction, the range of chromosome numbers in holocentric species does not appear remarkably different from those species with monocentric chromosomes. Although tolerance in fragmentation of chromosomes has been observed for some species with holocentric chromosomes, this evolution does not appear to lead to excessive ranges in chromosome number for many species (White 1977; Blackman 1980; Papeschi 1988, 1991; Brown, et al. 1992; Sunnucks, et al. 1996). An example of this is the order Lepidoptera, a group with holocentric chromosomes that contains large diversity in chromosome number (Wolf, et al. 1997). While a few species seem to be tolerant to chromosomal rearrangements (Brown, et al. 1992; Robinson 2017), many species exhibit little variation in chromosome number (White 1977; Emmel, et al. 1995; Robinson 2017). Though these observations have been made for some orders, patterns of chromosome number evolution driven by centromere type across large clades have yet to be investigated.

In this study, we used chromosome number and centromere type trait data for insects to test whether holocentric chromosomes have a higher rate of fusions and fissions (Figure 1). We chose to use insects because they are incredibly speciose and account for much of the variation present in animal species (Mora, et al. 2011). Using chromosome data, centromere data, and trees from previous studies, we fit a model of chromosome number evolution to our trait data using chromePLUS. This model of chromosome number evolution allows us to test the rate of chromosome number evolution in clades with holocentric and monocentric chromosomes to determine if there are significant differences in the rates. Our hypothesis is that clades with holocentric chromosomes will tolerate higher rates of fusions and fissions because the centromere is diffuse across the entire length of the chromosome, therefore each chromosome fragment will be more likely to properly segregate during meiosis.

**Methods**

We downloaded all available chromosome data for insects from a prior study (Blackmon, et al. 2017). This dataset is composed of 12,412 species comprising 376 families and 3,872 genera. The minimum haploid chromosome number is 2 while the maximum chromosome number is 141. There are 3,465 species with holocentric chromosomes and 8,946 species with monocentric chromosomes. For this dataset we collected the haploid chromosome number for each of the species. We additionally have obtained trees from a previous study that can be used for comparative analyses (Church, et al. 2019). We have downloaded two sets of phylogenetic trees based on different backbone trees that will be used for our comparative analysis. Each phylogeny includes 1,726 genera and contains a sample of 100 trees from a posterior distribution. Both of these distributions of trees are to the genera-level and matching this data to our trait dataset we have an overlap of 599 tips (Figure 1). We are fitting our model on each tree from the posterior distribution and we randomly sample trait data when more than one species is available for a genus. This approach allows us to account for uncertainty in the phylogeny and tip states.

Using the trait data and the posterior distribution trees, we implemented a chromosome number evolution model using chromePlus (Blackmon, et al. 2019). This model allows us to determine if the rate of chromosome number evolution is significantly different in clades with holocentric and monocentric chromosomes. We obtained estimates of six parameters: rates of chromosome number increase, fissions, (γ1 and γ2), rates of chromosome number decrease, fusions, (δ1 and δ2), and rates of change in karyotype state, monocentric vs. holocentric (*q*12 and *q*21). We then used an uninformative, unbounded improper prior that assumed that all non-negative values are equally likely for all of the parameters. The Markov Chain Monte Carlo (MCMC) was initialized with parameter values drawn from a uniform distribution from 0 to 8, which is broad but biologically reasonable. Preliminary analysis indicated that MCMC chains reached convergence, however some were sampling non-biologically relevant regions of parameter space. To fix this problem, we added a prior that drew from an exponential distribution with a shape parameter of 0.5. This prior tightened our sampled parameter space and ensured that values that were outside of a biologically relevant region were penalized. We repeated the MCMC with all 100 trees at 50 generations each. We removed the first twenty-five samples as our burnin for each run.

We repeated similar analysis as above for the analysis of orders, however we ran the data twice. Once with polyploidy and once without polyploidy included in the model. We only included orders with more than 20 genera in the analysis. This was to ensure that we had a large enough sample size as well as enough time along the branches to determine the rates of chromosome evolution. In total, we used ten orders for our analysis. Three of which had holocentric chromosomes, and seven that had monocentric chromosomes. The three orders with holocentric chromosomes were Hemiptera, Lepidoptera, and Odonata. We used the same prior as above from an exponential distribution with a shape parameter of 0.5 and initialized our MCMC with parameter values drawn from a uniform distribution from either 0-2 or 3 depending on if we included polyploidy in our analysis. We repeated the MCMC with all 100 trees at 50 generations each. We removed the first twenty-five samples as our burnin for each run.

**Results**

*Analysis of Monocentric and Holocentric Species*

We tested rates of chromosome evolution across the two insect trees. We included six parameters in our model as described in the methods section. These parameters included fusion, fission, and the transition between monocentric and holocentric chromosomes and vice versa. The data had a low variability and the rates did not show a difference between monocentric and holocentric chromosomes (Figure 2). This indicates that there are no significant differences among rates of chromosome evolution based on type of chromosome.

*Analysis of Orders*

We tested the rates of chromosome evolution including polyploidy for the ten orders that contained more than 20 samples for analysis. The range in rates was relatively low and there were no trends in the data (Figure 3a). The addition of polyploidy in the model decreased the variation of all rates of chromosome evolution. The fusion rate, however, was higher in Blattodea, Isoptera, and Phasmatodea, but this does not include all of the monocentric species. The holocentric orders had similar rates to all other orders.

Next, we tested the rates of chromosome evolution without polyploidy for the ten orders. There was more variability in this data, however, there were still no clear trends in the data (Figure 3b). Lepidoptera, however, had an increase in both the fission and fusion rates as compared to the rates with polyploidy. This is most likely driven by large variation in chromosome number associated with the order. The other species that were also holocentric do not show an increase in fission and fusion rates like Lepidoptera. This indicates differences among orders, but no relationship between holocentricity and rates of chromosome evolution.

**Discussion**

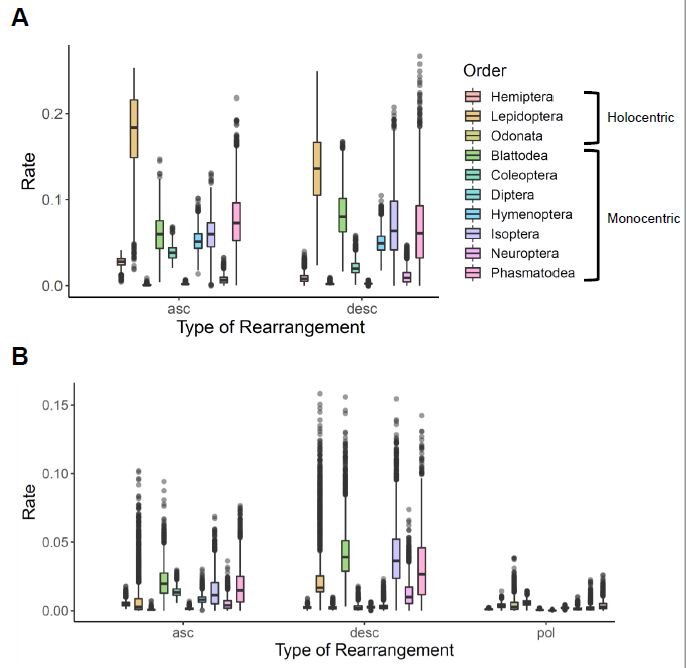
The hypothesis that holocentric chromosomes are more tolerant to fusions and fissions and therefore have a higher rate of chromosome evolution was not supported by our data. Overall, the rates of chromosome evolution were similar among monocentric and holocentric species. When our data was separated based on orders, the rates showed differences among orders and not due to differences in chromosome type. This is most likely due differences amongst orders based on different selective pressures experienced by the clades rather than the type of chromosome. Holocentric chromosomes may be more tolerant to fusions and fissions. However, chromosome number is constrained by external factors that have not caused a difference in the rate of chromosome evolution due to holocentricity alone.

**Figures:**



**Figure 1: Phylogeny of type of centromeres and chromosome number.** The black branches represent orders with monocentric chromosomes and the gray branches represent orders with holocentric chromosomes. The colored circles at the tips represents the haploid chromosome number the color scale is log transformed to allow better visualization of variation in species with low chromosome number.

**Figure 2: Rates of chromosome evolution.**



**Figure 3:** **Rate of chromosome evolution based on order**. Ascending rates represent the rate of fissions, descending rates represent the rate of fusions, and pol rates represent the rate of polyploidy. (A) Modelling for rates of chromosome evolution with polyploidy. The addition of polyploidy in the model has decreased the variation of all rates of chromosome evolution. There is no trend between holocentric and monocentric chromosome orders. (B) Modelling for the rates of chromosome evolution without polyploidy. The removal of polyploidy has kept most of the other species at about the same rates of chromosome evolution, expect for Lepidoptera. Lepidoptera has an increase in both fusion and fission rates.

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